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PRESERVATION OF HUMAN SERUM FOR WASSERMANN REACTION [1]

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Serologists, and laboratory workers in general, whose task it is to perform the Wassermann reaction for syphilis, are frequently annoyed with specimens that are utterly unfit for examination. The physician, many miles from the laboratory, collects the specimen without the necessary aseptic precautions, puts it into an unsterilized bottle, and sends it to the laboratory, where it arrives a week or so later and is nothing but a mass of putrid blood. Many methods for conducting the Wassermann reaction have been proposed, but little or no attention has been paid to the collection and the preparation of the specimens to be examined.

The investigation here to be reported was undertaken with the view of finding a suitable chemical agent that would render the serum sterile and keep it sterile without interfering with the test to be conducted. Of chemical agents, phenol, lysol, tricresol, chloroform, formalin, and glycerin were tried. Phenol, lysol, tricresol, and chloroform were soon abandoned because of the precipitate they produced when added to serum in quantities large enough to destroy microörganisms. Formalin and glycerin were suitable in this respect and were tested to some extent.

Preparation of the specimen.—About 10 cubic centimeters of physiologic salt solution were put into each of a number of suitable test tubes. The tubes were stoppered with cotton and were sterilized in the autoclave. The sterilized tubes with the salt solution were stored until needed. Salt solution was put into the tubes to prevent the blood clot from adhering to the wall of the tube, which frequently happens when blood is put into a dry tube. Immediately before use the salt solution was poured out of the test tube, and the blood was put in by letting it flow down the inside of the tube to prevent frothing. The

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tube with the blood was left at room temperature for two or three hours to allow the blood to clot. If the clot adhered to the tube and did not sink in the serum, it was loosened with a sterile wire and was pushed down into the serum. The specimen was now put into the refrigerator for from twelve to twenty-four hours to allow the serum to separate and the loose corpuscles to settle to the bottom of the tube. After the corpuscles had settled, the clear serum was pipetted off and was mixed with the preservative, after which it was tested at suitable intervals.

Antigen.—The antigen used was plain alcoholic extract of human heart-muscle, and about one fourth the anticomplementary dose was used per tube in the test.

Complement.—As alien complement the pooled sera of three guinea pigs were used in quantities of 0.1, 0.05, and 0.025 cubic centimeter.

Hæmolytic amboceptor.—As artificial hæmolytic amboceptor the antihuman amboceptor advocated by Noguchi was used in doses of 1 to 1.5 unit per tube. The smallest quantity of amboceptor that with 0.05 cubic centimeter of complement dissolved the test dose of corpuscles in one hour was called a unit.

Corpuscles.—Human corpuscles were well washed, and 0.5 cubic centimeter of a 4 per cent suspension in physiologic salt solution was used per tube.

Glassware.—Two sets of test tubes were used—one set as antigen tubes and another set as control tubes. Test tubes that had once been used as antigen tubes were never used as control tubes. Other glassware, such as pipettes, graduates, beakers, and flasks, that was used for serum was used for serum only, and that which was used for antigen was used for antigen only.

Methods.—Two methods of conducting the serum test for syphilis were used—namely, the Wassermann method with human hæmolytic system and the method described by Tschernogubow,[2] Hecht,[3] Gurd,[4] and others. As human serum as a rule dissolves sheep corpuscles better than the corpuscles of the guinea pig, sheep corpuscles were used, as first advocated by Tschernogubow.

Technique of conducting the Wassermann reaction.—Unless unheated serum was tested for anticomplementary properties, the serum was heated to between 55° C. and 56° C. for thirty minutes before testing. Six test tubes, three, designated as 1, 2, and 3 antigen tubes, and three, designated as 1′, 2′, and 3′

[2] *Deut. med. Wochenschr.* (1909), **35**, 668.
[3] *Wien. klin. Wochenschr.* (1909), **22**, 338.
[4] *Journ. Infect. Dis.* (1911), **8**, 427.

control tubes, were used. Of the serum to be tested, 0.6 cubic centimeter was diluted with 2.4 cubic centimeters of physiologic salt solution, and 0.5 cubic centimeter of the diluted serum was put into each of the six test tubes. Each tube of the first pair, tubes 1 and 1′, received 0.5 cubic centimeter of 1:5 dilution of complement serum. Of the second pair of tubes, tubes 2 and 2′, each received 0.5 cubic centimeter of 1:10 dilution of complement serum, and each of the third pair of tubes, tubes 3 and 3′, received 0.5 cubic centimeter of 1:20 dilution of complement serum. Each antigen tube received 0.5 cubic centimeter of diluted antigen representing about one fourth of the anticomplementary dose, and to each of the control tubes 0.5 cubic centimeter of physiologic salt solution was added. Now the tubes were placed in the incubator at 37° C. for one hour. After an hour in the incubator 1 cubic centimeter of sensitized corpuscles was added to each tube; the tubes were well shaken; returned to the incubator for one hour, during which time they were shaken at least three times; were removed to room temperature; and the results were read about three hours after the corpuscles had been added.

Technique of conducting the Tschernogubow modification of the Wassermann reaction.—This method utilizes complement and hæmolytic amboceptor normally present in human serum. When fresh serum was tested, the complement and hæmolytic amboceptor were derived from the serum tested, while old serum, heated or unheated, was reactivated with normal human serum.

Ten test tubes, five antigen tubes marked 1, 2, 3, 4, and 5 and five control tubes marked 1′, 2′, 3′, 4′, and 5′, were used for each test. If the serum to be tested was fresh and unheated, 1.6 cubic centimeters of serum were diluted to 4 cubic centimeters with physiologic salt solution. Each tube of the first pair, tubes 1 and 1′, received 1 cubic centimeter of diluted serum representing 0.4 cubic centimeter of serum. The remaining 2 cubic centimeters of diluted serum were further diluted with 2 cubic centimeters of physiologic salt solution, and 1 cubic centimeter of this dilution was put into each of the second pair of tubes, tubes 2 and 2′. Each of the second pair of tubes received 0.2 cubic centimeter of serum. These dilutions were continued until all of the five pairs of tubes were supplied with diluted serum. The quantities of serum represented in the tubes were 0.4, 0.2, 0.1, 0.05, and 0.025 cubic centimeter. Each of the antigen tubes received 1 cubic centimeter of diluted antigen representing about one fourth of the anticomplementary dose, and each control tube received 1 cubic centimeter of physiologic

salt solution. The tubes were put into the incubator at 37° C. for one hour. After having been in the incubator one hour, 1 cubic centimeter of 2 per cent suspension of sheep corpuscles was added to each tube and each tube was well shaken. The tubes were returned to the incubator for one hour and were then removed to room temperature; the results were read about three hours after the corpuscles had been added.

With old serum, or with heated serum, the test was conducted in the following manner: One cubic centimeter of serum was diluted to 5 cubic centimeters with physiologic salt solution, and 0.5 cubic centimeter of the diluted serum was put into each of the ten test tubes. Normal human serum diluted to 0.5 cubic centimeter was added as in the test just described for fresh serum. Antigen and corpuscles were used as already described.

TEST 1

Serum preserved by formalin tested by the Wassermann method.—Specimens 4318, 4319, 4326, and 4328 were secured on September 29, 1915, and the sera were drawn off the clots on September 30, 1915. Each serum was divided into two portions, A and B. Portion A was tested by the Wassermann method without having been mixed with formalin. Portion B was mixed with an equal volume of 1:500 solution of formalin in physiologic salt solution, was kept at room temperature in a cork-stoppered test tube, and was tested at intervals of about a week. A bacteriologic test was made of each serum.

TABLE I.—*The influence of formalin on the Wassermann reaction.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Serum.	Amboceptor.	Tube—						Result.
							1	2	3	1′	2′	3′	
	1915.		1915.	1915.	*cc.*	*Unit.*							
4318	Sept. 29	A	Sept. 30	Sept. 30	0.1	1.0	+	+	tr	+	+	tr	Negative.
		B	Oct. 7	Oct. 7	0.1	1.0	+	+	tr	+	+	tr	Do.
		B	Oct. 12	Oct. 12	0.1	1.0	+	±	0	+	±	0	Do.
4319	...do.....	A	Sept. 30	Sept. 30	0.1	1.0	+	+	tr	+	+	tr	Do.
		B	Oct. 7	Oct. 7	0.1	1.0	+	+	tr	+	+	tr	Do.
		B	Oct. 12	Oct. 12	0.1	1.0	+	tr	0	+	tr	0	Do.
4326	...do.....	A	Sept. 30	Sept. 30	0.1	1.0	+	0	0	+	+	tr	Strongly positive.
		B	Oct. 7	Oct. 7	0.1	1.0	+	+	tr	+	+	tr	Negative.
		B	Oct. 12	Oct. 12	0.1	1.0	+	tr	0	+	tr	0	Do.
4328	...do.....	A	Sept. 30	Sept. 30	0.1	1.0	+	0	0	+	+	tr	Strongly positive.
		B	Oct. 7	Oct. 7	0.1	1.0	+	+?	0	+	+	0	Faintly positive.
		B	Oct. 12	Oct. 12	0.1	1.0	+	tr?	0	+	tr	0	Do.

The sign + means complete hæmolysis; ±, hæmolysis between 50 per cent and 100 per cent; tr (trace), hæmolysis less than 50 per cent; +?, barely perceptible sediment; ±? in antigen tube and ± in corresponding control tube, or tr? in antigen tube and tr in corresponding control tube, means very slight difference.

Table I shows that sera 4318 and 4319, which gave negative results with the Wassermann reaction before formalin was added, also gave negative results after formalin had been added. Serum 4326, which before formalin was added gave a strong positive result, gave a negative result on the seventh day and on the twelfth day after the formalin had been added. Serum 4328 gave a strong positive result before formalin was added and a faintly positive result on the seventh day and on the twelfth day after the formalin had been added. All sera were bacteriologically sterile.

TEST 2

Serum preserved by formalin tested by the Wassermann method.—Specimens 4401, 4403, 4404, and 4405 were secured on October 29, 1915. The sera were drawn off the clots on October 30, 1915. Each serum was divided into two portions, A and B. Portion A received no formalin; it was tested by the Wassermann method and by the Tschernogubow modification of the Wassermann reaction on October 30, 1915. Portion B was mixed with an equal volume of 1:500 solution of formalin in physiologic salt solution and was tested on November 7, 1915, by the Wassermann method and by the Tschernogubow modification of the Wassermann reaction before and after it had been heated. Each serum was examined bacteriologically.

TABLE II.—*The influence of formalin on the Wassermann reaction.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Serum.	Ambocceptor.	Tube— 1	2	3	1′	2′	3′	Result.
	1915.		1915.	1915.	*cc.*	*Unit.*							
4401	Oct. 29	A	Oct. 30	Oct. 30	0.1	1.0	+	+	0	+	+	tr	Weakly positive.
		B	.....	Nov. 7	0.1	1.0	+	tr	0	+	±	0	Do.
		B	Nov. 7	...do	0.1	1.0	+	±	0	+	±	0	Negative.
4403	...do	A	Oct. 30	Oct. 30	0.1	1.0	±	0	0	+	+	tr	Strongly positive.
		B	.....	Nov. 7	0.1	1.0	tr	0	0	±	0	0	Weakly positive.
		B	Nov. 7	...do	0.1	1.0	+	tr	0	+	tr	0	Negative.
4404	...do	A	Oct. 30	Oct. 30	0.1	1.0	+	tr	0	+	+	0	Moderately positive.
		B	.....	Nov. 7	0.1	1.0	tr	0	0	±	0	0	Weakly positive.
		B	Nov. 7	...do	0.1	1.0	+	tr	0	+	tr	0	Negative.
4405	...do	A	Oct. 30	Oct. 30	0.1	1.0	+	±	0	+	+	0	Weakly positive.
		B	.....	Nov. 7	0.1	1.0	tr	0	0	±	0	0	Do.
		B	Nov. 7	...do	0.1	1.0	+	tr	0	+	tr	0	Negative.

TABLE II'.—*The influence of formalin on the Tschernogubow modification of the Wassermann reaction.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Serum.	Tube—										Result.
						1	2	3	4	5	1'	2'	3'	4'	5'	
	1915.		1915.	1915.	*cc.*											
4401	Oct. 29	A	.....	Oct. 30	.....	+	0	0	0	0	+	+	tr	0	0	Moderately positive.
		B	.....	Nov. 7	0.1	+	+	±	0	0	+	+	+	0	0	Weakly positive.
		B	Nov. 7	...do	0.1	+	+	+	tr	0	+	+	+	tr	0	Negative.
4403	...do	A	.....	Oct. 30	.....	0	0	0	0	0	+	+	+	0	0	Strongly positive.
		B	.....	Nov. 7	0.1	+	+	±	0	0	+	+	+	0	0	Weakly positive.
		B	Nov. 7	...do	0.1	+	+	+	0	0	+	+	+	0	0	Negative.
4404	...do	A	.....	Oct. 30	.....	+	0	0	0	0	+	+	+	0	0	Strongly positive.
		B	.....	Nov. 7	0.1	+	+	+	0	0	+	+	+	tr	0	Weakly positive.
		B	Nov. 7	...do	0.1	+	+	±	0	0	+	+	+	0	0	Do.
4405	...do	A	.....	Oct. 30	.....	+	+	0	0	0	+	+	+	0	0	Moderately positive.
		B	.....	Nov. 7	0.1	+	+	±	0	0	+	+	+	0	0	Weakly positive.
		B	Nov. 7	...do	0.1	+	+	+	0	0	+	+	+	0	0	Negative.

Tables II and II' show the results obtained by testing formalinized serum by the Wassermann method and by the Tschernogubow modification. Some of these sera gave strongly positive results before formalin had been added. With the unheated sera weakly positive results were obtained a week after the formalin had been added. After having been heated to 55° C. for thirty minutes, all but one serum gave negative results. Unheated, these sera were but slightly anticomplementary a week after having been secured; less so for the Tschernogubow modification than for the Wassermann method. All sera were bacteriologically sterile.

TEST 3

The influence of glycerin on the Wassermann reaction.—Specimens 4318, 4319, 4320, 4321, 4322, 4326, 4328, 4335, 4336, and 4338 were secured on September 29, 1915. On September 30, 1915, the sera were drawn off the clots. Each serum was divided into two portions, A and B. Portion A was tested by the Wassermann method on September 30, 1915, without having been mixed with glycerin. Portion B was mixed with an equal volume of sterilized, neutral glycerin, was kept at room temperature in a cork-stoppered test tube, and was tested at intervals of a week or more. Each serum was examined for bacterial contamination.

TABLE III.—*The influence of glycerin on the Wassermann reaction.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Quantity of serum.	Amboceptor.	Tube— 1	2	3	1′	2′	3′	Result.
	1915.		1915.	1915.	*cc.*	*Unit.*							
		A	Sept. 30	Sept. 30	0.1	1.25	+	+	±	+	+	±	Negative.
		B	Oct. 7	Oct. 7	0.1	1.25	+	+	tr	+	+	tr	Do.
4318	Sept. 29	B	Oct. 14	Oct. 14	0.1	1.25	+	±	0	+	±	0	Do.
		B	Oct. 24	Oct. 24	0.1	1.25	+	±	0	+	±	0	Do.
		B	----------	---do ----	0.1	1.25	0	0	0	0	0	0	Anticomplementary.
		A	Sept. 30	Sept. 30	0.1	1.25	+	+	±	+	+	±	Negative.
		B	Oct. 7	Oct. 7	0.1	1.25	+	+	tr	+	+	tr	Do.
4319	---do ----	B	Oct. 14	Oct. 14	0.1	1.25	+	±	0	+	±	0	Do.
		B	Oct. 24	Oct. 24	0.1	1.25	+	tr	0	+	tr	0	Do.
		B	----------	---do ----	0.1	1.25	0	0	0	0	0	0	Anticomplementary.
		A	Sept. 30	Sept. 30	0.1	1.25	+	+	tr	+	+	tr	Negative.
		B	Oct. 7	Oct. 7	0.1	1.25	+	+	±	+	+	±	Do.
4320	---do ----	B	Oct. 14	Oct. 14	0.1	1.25	+	+	0	+	+	0	Do.
		B	Oct. 24	Oct. 24	0.1	1.25	+	±	0	+	±	0	Do.
		B	----------	---do ----	0.1	1.25	0	0	0	0	0	0	Anticomplementary.
		A	Sept. 30	Sept. 30	0.1	1.25	+	+	±	+	+	±	Negative.
		B	Oct. 7	Oct. 7	0.1	1.25	+	+	tr	+	+	tr	Do.
4321	---do ----	B	Oct. 14	Oct. 14	0.1	1.25	+	+	0	+	+	0	Do.
		B	Oct. 24	Oct. 24	0.1	1.25	+	±	0	+	±	0	Do.
		B	----------	---do ----	0.1	1.25	0	0	0	0	0	0	Anticomplementary.
		A	Sept. 30	Sept. 30	0.1	1.25	+	+	±	+	+	±	Negative.
		B	Oct. 7	Oct. 7	0.1	1.25	+	+	±	+	+	±	Do.
4322	---do ----	B	Oct. 14	Oct. 14	0.1	1.25	+	+	0	+	+	0	Do.
		B	Oct. 24	Oct. 24	0.1	1.25	+	±	0	+	±	0	Do.
		B	----------	---do ----	0.1	1.25	0	0	0	0	0	0	Anticomplementary.
		A	Sept. 30	Sept. 30	0.1	1.25	+	0	0	+	+	tr	Strongly positive.
		B	Oct. 7	Oct. 7	0.1	1.25	±	0	0	+	+	0	Do.
4326	---do ----	B	Oct. 14	Oct. 14	0.1	1.25	0	0	0	+	tr	0	Do.
		B	Oct. 24	Oct. 24	0.1	1.25	0	0	0	+	tr	0	Do.
		B	----------	---do ----	0.1	1.25	0	0	0	0	0	0	Anticomplementary.
		A	Sept. 30	Sept. 30	0.1	1.25	+	±	0	+	+	±	Strongly positive.
		B	Oct. 7	Oct. 7	0.1	1.25	+	tr	0	+	+	tr	Do.
4328	---do ----	B	Oct. 14	Oct. 14	0.1	1.25	tr	0	0	+	tr	0	Do.
		B	Oct. 24	Oct. 24	0.1	1.25	tr	0	0	+	±	0	Do.
		B	----------	---do ----	0.1	1.25	0	0	0	0	0	0	Anticomplementary.
		A	Sept. 30	Sept. 30	0.1	1.25	+	±	0	+	+	tr	Moderately positive.
		B	Oct. 7	Oct. 7	0.1	1.25	+	±	0	+	+	tr	Do.
4335	---do ----	B	Oct. 14	Oct. 14	0.1	1.25	tr	0	0	+	tr	0	Strongly positive.
		B	Oct. 24	Oct. 24	0.1	1.25	tr	0	0	+	tr	0	Do.
		B	----------	---do ----	0.1	1.25	0	0	0	0	0	0	Anticomplementary.

TABLE III.—*The influence of glycerin on the Wassermann reaction*—Continued.

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Quantity of serum.	Ambocceptor.	Tube—						Result.
							1	2	3	1′	2′	3′	
	1915.		1915.	1915.	*cc.*	*Unit.*							
		A	Sept. 30	Sept. 30	0.1	1.25	+	+	0	+	+	±	Moderately positive.
		B	Oct. 7	Oct. 7	0.1	1.25	+	±	0	+	+	tr	Do.
4336	...do....	B	Oct. 14	Oct. 14	0.1	1.25	tr	0	0	+	tr	0	Strongly positive.
		B	Oct. 24	Oct. 24	0.1	1.25	tr	0	0	+	tr	0	Do.
		B	.....	...do....	0.1	1.25	0	0	0	0	0	0	Anticomplementary.
		A	Sept. 30	Sept. 30	0.1	1.25	+	±	0	+	+	±	Strongly positive.
		B	Oct. 7	Oct. 7	0.1	1.25	+	0	0	+	+	tr	Do.
4338	...do....	B	Oct. 14	Oct. 14	0.1	1.25	tr	0	0	+	±	0	Do.
		B	Oct. 24	Oct. 24	0.1	1.25	0	0	0	+	tr	0	Do.
		B	.....	...do....	0.1	1.25	0	0	0	0	0	0	Anticomplementary.

Table III shows the results obtained in testing glycerinated sera 4318, 4319, 4320, 4321, 4322, 4326, 4328, 4335, 4336, and 4338 by the Wassermann method. Glycerin did not noticeably influence this method. The results obtained by this test seven days, fourteen days, and twenty-four days after the sera had been mixed with glycerin were practically identical with the results obtained before the sera had been mixed with glycerin. It seems that sera that gave a positive result while fresh became more strongly positive with age. Negatives remained negative. Unheated, all sera were strongly anticomplementary on the twenty-fourth day after they had been mixed with glycerin; heating the sera to 55° C. for thirty minutes did not entirely destroy the anticomplementary property. All sera remained free from bacterial growth.

TEST 4

The influence of glycerin on the Wassermann reaction.—On October 13, 1915, specimens 4360, 4361, 4364, 4365, 4366, 4369, 4370, 4371, 4372, and 4374 were secured. The sera were drawn off the clots on October 14, 1915. Each serum was divided into two portions, A and B. Portion A, unglycerinated, was tested by the Wassermann method on October 14, 1915. Portion B was mixed with an equal volume of sterilized, neutral glycerin, was kept at room temperature in a cork-stoppered test tube, and was tested by the Wassermann method at intervals of about a week. A bacteriologic examination was made of each serum.

TABLE IV.—*The influence of glycerin on the Wassermann reaction.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Serum.	Amboceptor.	Tube—						Result.
							1	2	3	1′	2′	3′	
	1915.		1915.	1915.	*cc.*	*Unit.*							
		A	Oct. 14	Oct. 14	0.1	1.0	±	0	0	+	±	0	Strongly positive.
		B	Oct. 24	Oct. 24	0.1	1.0	±	0	0	+	±	0	Do.
4360	Oct. 13	B	----------	---do----	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
		B	Oct. 31	Oct. 31	0.1	1.0	tr	0	0	+	tr	0	Strongly positive.
		B	Nov. 7	Nov. 7	0.1	1.0	tr	0	0	+	tr	0	Do.
		A	Oct. 14	Oct. 14	0.1	1.0	+	0	0	+	+	tr	Do.
		B	Oct. 24	Oct. 24	0.1	1.0	0	0	0	+	tr	0	Do.
4361	---do----	B	----------	---do----	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
		B	Oct. 31	Oct. 31	0.1	1.0	0	0	0	+	tr	0	Strongly positive.
		A	Oct. 14	Oct. 14	0.1	1.0	+	tr	0	+	+	tr	Do.
		B	Oct. 24	Oct. 24	0.1	1.0	±	0	0	+	+	0	Do.
4364	---do----	B	----------	---do----	0.1	1.0	0	0	0	+	+	0	Do.
		B	Oct. 31	Oct. 31	0.1	1.0	tr	0	0	+	+	0	Do.
		B	Nov. 7	Nov. 7	0.1	1.0	tr	0	0	+	±	0	Do.
		A	Oct. 14	Oct. 14	0.1	1.0	+	+	tr	+	+	tr	Negative.
		B	Oct. 24	Oct. 24	0.1	1.0	+	±	0	+	±	0	Do.
4365	---do----	B	----------	---do----	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
		B	Oct. 31	Oct. 31	0.1	1.0	+	tr	0	+	tr	0	Negative.
		A	Oct. 14	Oct. 14	0.1	1.0	+	+	tr	+	+	tr	Do.
		B	Oct. 24	Oct. 24	0.1	1.0	+	±	0	+	±	0	Do.
4366	---do----	B	----------	---do----	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
		B	Oct. 31	Oct. 31	0.1	1.0	+	tr	0	+	tr	0	Negative.
		A	Oct. 14	Oct. 14	0.1	1.0	+	tr	0	+	+	tr	Strongly positive.
		B	Oct. 24	Oct. 24	0.1	1.0	tr	0	0	+	tr	0	Do.
4369	---do----	B	----------	---do----	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
		B	Oct. 31	Oct. 31	0.1	1.0	0	0	0	+	tr	0	Strongly positive.
		A	Oct. 14	Oct. 14	0.1	1.0	+	0	0	+	+	tr	Do.
		B	Oct. 24	Oct. 24	0.1	1.0	tr	0	0	+	tr	0	Do.
4370	---do----	B	----------	---do----	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
		B	Oct. 31	Oct. 31	0.1	1.0	0	0	0	+	0	0	Strongly positive.
		B	No. 7	Nov. 7	0.1	1.0	0	0	0	+	0	0	Do.
		A	Oct. 14	Oct. 14	0.1	1.0	+	+	tr	+	+	tr	Negative.
		B	Oct. 24	Oct. 24	0.1	1.0	+		0	+	±	0	Do.
4371	---do----	B	----------	---do----	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
		B	Oct. 31	Oct. 30	0.1	1.0	+	tr	0	+	tr	0	Negative.

TABLE IV.—*The influence of glycerin on the Wassermann reaction*—Continued.

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Serum.	Amboceptor.	Tube—						Result.
							1	2	3	1'	2'	3'	
	1915.		1915.	1915.	*cc.*	*Unit.*							
		A	Oct. 14	Oct. 14	0.1	1.0	+	+	0	+	+	±	Moderately positive.
		B	Oct. 24	Oct. 24	0.1	1.0	±	0	0	+	tr	0	Do.
4372	Oct. 13	B	----------	---do ----	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
		B	Oct. 31	Oct. 31	0.1	1.0	±	0	0	+	tr	0	Moderately positive.
		B	Nov. 7	Nov. 7	0.1	1.0	±	0	0	+	tr	0	Do.
		A	Oct. 14	Oct. 14	0.1	1.0	+	±	0	+	+	tr	Do.
		B	Oct. 24	Oct. 24	0.1	1.0	±	0	0	+	tr	0	Do.
		B	----------	---do ----	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
4374	---do ----	B	Oct. 31	Oct. 31	0.1	1.0	±	0	0	+	tr	0	Moderately positive.
		B	Nov. 7	Nov. 7	0.1	1.0	±	0	0	+	tr	0	Do.
4370	---do ----	B	---do ----	---do ----	0.1	1.5	±	0	0	+	+	0	Strongly positive.
4372	---do ----	B	---do ----	---do ----	0.1	1.5	+	tr	0	+	+	0	Moderately positive.
4374	---do ----	B	---do ----	---do ----	0.1	1.5	+	0	0	+	+	0	Strongly positive.

The result obtained with sera 4360, 4361, 4364, 4365, 4366, 4369, 4370, 4371, 4372, and 4374 by the Wassermann reaction are shown in Table IV. The glycerinated portion of each of these sera gave results practically identical with the result obtained with the nonglycerinated portion. Unheated, nine of these ten sera were strongly anticomplementary on the tenth day, after they had been mixed with glycerin. One serum, No. 4364, had not become anticomplementary. The anticomplementary property was not entirely destroyed by heating the sera to 55° C. for thirty minutes, and it did not alter the result of the test. Such anticomplementary property was overcome by increasing the quantity of amboceptor. All sera were sterile.

TEST 5

The influence of glycerin on the Tschernogubow modification of the Wassermann reaction.—Specimens 4361, 4365, 4366, and 4369 were secured on October 13, 1915. The sera were drawn off the clots on October 14, 1915. Each serum was divided into two portions, A and B. Unheated and unglycerinated, portion A was tested on October 14, 1915. Portion B was mixed with an equal volume of sterilized, neutral glycerin, was kept at room temperature in a cork-stoppered test tube, and was tested two weeks later.

TABLE V.—*The influence of glycerin on the Tschernogubow modification.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Serum.	Tube—										Result.
						1	2	3	4	5	1′	2′	3′	4′	5′	
	1915.		1915.	1915.	cc.											
4361	Oct. 13	A	----------	Oct. 14	-------	0	0	0	0	0	+	+	+	0	0	Strongly positive.
		B	Oct. 31	Oct. 31	0.1	0	0	0	0	0	+	+	tr	0	0	Do.
4365	---do ----	A	----------	Oct. 14	-------	+	+	±	0	0	+	+	±	0	0	Negative.
		B	Oct. 31	Oct. 31	0.1	+	+	+	0	0	+	+	+	0	0	Do.
4366	---do ----	A	----------	Oct. 14	-------	+	+	+	0	0	+	+	+	0	0	Do.
		B	Oct. 31	Oct. 31	0.1	+	+	+	0	0	+	+	+	0	0	Do.
4369	---do ----	A	----------	Oct. 14	-------	0	0	0	0	0	+	+	+	±	0	Strongly positive.
		B	Oct. 31	Oct. 31	0.1	+	0	0	0	0	+	+	+	0	0	Do.

Table V shows that with these four sera glycerin did not influence the Tschernogubow modification of the Wassermann reaction.

TEST 6

The influence of glycerin on the Wassermann reaction.—Specimens 4394, 4395, 4396, 4397, 4398, and 4399 were secured on October 27, 1915, and the sera were drawn off the clots the next day. Each serum was divided into four portions—A, B, C, and D. Unglycerinated, portion A was heated and tested by the Wassermann method on October 28, 1915. Portion B was mixed with an equal volume of sterilized, neutral glycerin and was heated to 55° C. for thirty minutes on October 28, 1915; was tested by the Wassermann method on November 7, 1915. Portion C was mixed with an equal volume of sterilized, neutral glycerin on October 28, 1915, and was heated and tested by the Wassermann method on November 7, 1915. D was mixed with an equal volume of sterilized, neutral glycerin on October 28, 1915, and without having been heated, was tested by the Wassermann method on November 7, 1915. Each serum was examined bacteriologically.

Table VI shows the results obtained with glycerinated sera 4394, 4395, 4396, 4397, and 4398, tested by the Wassermann method. The glycerin did not noticeably influence the Wassermann reaction. Glycerinated sera that were heated on October 28, 1915, and were tested on November 7, 1915, were not more anticomplementary than were sera that were heated and tested on November 7, 1915. Unheated, five of the six sera were strongly anticomplementary on November 7, 1915. Serum 4399 was no more anticomplementary before having been heated than it was after it had been heated on November 7, 1915.

TABLE VI.—*The influence of glycerin on the Wassermann reaction.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Serum.	Amboceptor.	Tube— 1	2	3	1′	2′	3′	Result.
	1915.		1915.	1915.	*cc.*	*Unit.*							
		A	Oct. 28	Oct. 28	0.1	1.0	+	0	0	+	±	0	Moderately positive.
4394	Oct. 27	B	---do ----	Nov. 7	0.1	1.0	±	0	0	+	tr	0	Do.
		C	Nov. 7	---do ----	0.1	1.0	tr	0	0	±	tr	0	Do.
		D	----------	---do ----	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
		A	Oct. 28	Oct. 28	0.1	1.0	+	±	0	+	±	0	Negative.
		B	---do ----	Nov. 7	0.1	1.0	+	tr	0	+	tr	0	Do.
4395	---do ----	C	Nov. 7	---do ----	0.1	1.0	+	tr	0	+	tr	0	Do.
		D	----------	---do ----	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
		A	Oct. 28	Oct. 28	0.1	1.0	+	0	0	+	+	0	Strongly positive.
		B	---do ----	Nov. 7	0.1	1.0	tr	0	0	+	tr	0	Do.
4396	---do ----	C	Nov. 7	--- do----	0.1	1.0	tr	0	0	+	tr	0	Do.
		D	----------	---do ----	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
		A	Oct. 28	Oct. 28	0.1	1.0	tr	0	0	+	±	0	Strongly positive.
4397	---do ----	B	---do ----	Nov. 7	0.1	1.0	0	0	0	+	tr	0	Do.
		C	Nov. 7	---do ----	0.1	1.0	0	0	0	+	0	0	Do.
		D	----------	---do ----	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
		A	Oct. 28	Oct. 28	0.1	1.0	±	0	0	+	±	0	Strongly positive.
4398	---do ----	B	---do ----	Nov. 7	0.1	1.0	tr	0	0	+	tr	0	Do.
		C	Nov. 7	---do ----	0.1	1.0	tr	0	0	+	tr	0	Do.
		D	----------	---do ----	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
		A	Oct. 28	Oct. 28	0.1	1.0	+	±	0	+	±	0	Negative.
4399	---do ----	B	---do ----	Nov. 7	0.1	1.0	+	tr	0	+	tr	0	Do.
		C	Nov. 7	---do ----	0.1	1.0	+	tr	0	+	tr	0	Do.
		D	----------	---do ----	0.1	1.0	+	tr	0	+	tr	0	Do.

TEST 7

The influence of glycerin on the Tschernogubow modification of the Wassermann reaction.—Specimens 4394, 4395, and 4396 were secured on October 27, 1915. The sera were drawn off the clots on October 28, 1915. Each serum was divided into three portions—A, B, and C. Portion A, unglycerinated and unheated, was tested by the Tschernogubow modification on October 28, 1915. Portion B was heated to 55° C. for thirty minutes and was then mixed with an equal volume of sterilized, neutral glycerin. On November 7, 1915, portion B was subdivided into portions B and B′. Portion B was tested without further heating, and portion B′ was tested after it had been reheated. Unheated, portion C was mixed with an equal volume of sterilized, neutral glycerin on October 28, 1915. On Novem-

ber 7, 1915, portion C was subdivided into two portions, C and C′. Portion C was tested without having been heated, and portion C′ was tested after it had been heated to 55° C. for thirty minutes.

TABLE VII.—*The influence of glycerin on the Tschernogubow modification of the Wassermann reaction.*

No. of serum.	Date secured.	Portion.	Date heated.	Date reheated.	Date tested.	Serum.	Tube— 1	2	3	4	5	1′	2′	3′	4′	5′	Result.
	1915.		1915.	1915.	1915.	cc.											
		A	----	----	Oct. 28	----	+	0	0	0	0	+	+	+	0	0	Moderately positive.
		B	Oct. 28	----	Nov. 7	0.1	+	tr	0	0	0	+	+	tr	0	0	Do.
4394	Oct. 27	B′	..do...	Nov. 7	..do...	0.1	+	tr	0	0	0	+	+	±	0	0	Do.
		C	----	----	..do...	0.1	tr	0	0	0	0	+	tr	0	0	0	Do.
		C′	Nov. 7	----	..do...	0.1	+	tr	0	0	0	+	+	±	0	0	Do.
		A	----	----	Oct. 28	----	+	+	+	0	0	+	+	+	0	0	Negative.
		B	Oct. 28	----	Nov. 7	0.1	+	+	tr	0	0	+	+	tr	0	0	Do.
4395	..do...	B′	..do...	Nov. 7	..do...	0.1	+	+	±	0	0	+	+	±	0	0	Do.
		C	----	----	..do...	0.1	+	tr	0	0	0	+	tr	0	0	0	Do.
		C′	Nov. 7	----	..do...	0.1	+	+	±	0	0	+	+	±	0	0	Do.
		A	----	----	Oct. 28	----	+	0	0	0	0	+	+	+	+	0	Strongly positive.
		B	Oct. 28	----	Nov. 7	0.1	+	0	0	0	0	+	+	±	0	0	Do.
4396	..do...	B′	..do...	Nov. 7	..do...	0.1	±	0	0	0	0	+	+	±	0	0	Do.
		C	----	----	..do...	0.1	0	0	0	0	0	+	±	0	0	0	Do.
		C′	Nov. 7	----	..do...	0.1	+	0	0	0	0	+	+	±	0	0	Do.

As is shown in Table VII, glycerin did not influence the Tschernogubow modification of the Wassermann reaction. Heating the sera to 55° C. for thirty minutes more than once did not alter the results obtained, and unheated, these sera had become but slightly anticomplementary for the Tschernogubow modification of the Wassermann reaction in eleven days.

TEST 8

The influence of glycerin on the Tschernogubow modification of the Wassermann reaction.—Specimens 4397, 4398, and 4399 were secured on October 27, 1915. On the next day the sera were drawn off the clots. Each serum was divided into two portions, A and B. Unglycerinated and unheated, portion A was tested by the Tschernogubow modification of the Wassermann reaction on October 28, 1915. Portion B was mixed with an equal volume of sterilized, neutral glycerin and was kept in a cork-stoppered test tube at room temperature. On November 7, 1915, portion B was tested by the Tschernogubow modification before and after the serum had been heated to 55° C. for thirty minutes.

TABLE VIII.—*The influence of glycerin on the Tschernogubow modification of the Wassermann reaction.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Serum.	Tube—										Result.
						1	2	3	4	5	1′	2′	3′	4′	5′	
	1915.		1915.	1915.	*cc.*											
		A	.....	Oct. 28	.....	0	0	0	0	0	+	+	+	+	0	Strongly positive.
4397	Oct. 27	B	.....	Nov. 7	0.1	0	0	0	0	0	+	+	0	0	0	Do.
		B	Nov. 7	...do	0.1	±	0	0	0	0	+	+	±	0	0	Do.
		A	.....	Oct. 28	.....	0	0	0	0	0	+	+	+	0	0	Do.
4398	...do	B	.....	Nov. 7	0.1	±	0	0	0	0	+	+	0	0	0	Do.
		B	Nov. 7	...do	0.1	+	+	tr	0	0	+	+	+	±	0	Do.
		A	.....	Oct. 28	.....	+	+	±	0	0	+	+	±	0	0	Negative.
4399	...do	B	.....	Nov. 7	0.1	+	±	0	0	0	+	±	0	0	0	Do.
		B	Nov. 7	...do	0.1	+	+	±	0	0	+	+	±	0	0	Do.

Table VIII shows that in test 8 the glycerin did not influence the Tschernogubow modification of the Wassermann reaction. Unheated, these three sera were moderately anticomplementary on November 7, 1915. Heating the sera to 55° C. for thirty minutes destroyed the anticomplementary property.

TEST 9

The appearance of anticomplementary properties.—Specimens 4557, 4558, 4559, 4560, and 4561 were secured on January 4, 1916. On January 5, 1916, the sera were drawn off the clots. Each serum was divided into seven portions—A, B, C, D, E, F, and G. Unglycerinated, portion A was tested by the Wassermann method on January 5, 1916. Portion B was heated to 55° C. for thirty minutes, was mixed with an equal volume of sterilized, neutral glycerin, and was kept at room temperature in a cork-stoppered test tube. Portions C, D, E, F, and G were mixed with equal volumes of sterilized, neutral glycerin and were kept at room temperature in cork-stoppered test tubes. Beginning with January 5, 1916, one portion of glycerinated serum was heated to 55° C. for thirty minutes on every third day until all the portions had been heated. Portion C was heated on January 5; portion D, on January 8; portion E, on January 11; portion F, on January 14; and portion G, on January 16, 1916. On the last day portions B, C, D, E, F, and G were tested by the Wassermann method and a bacteriologic test was made of each serum.

TABLE IX.—*The appearance of anticomplementary properties.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Serum.	Amboceptor.	Tube— 1	2	3	1′	2′	3′	Result.
	1916.		1916.	1916.	*cc.*	*Unit.*							
		A	Jan. 5	Jan. 5	0.1	1.0	+	+	tr	+	+	tr	Negative.
		B	---do----	Jan. 16	0.1	1.0	+	+	0	+	+	0	Do.
		C	---do----	---do----	0.1	1.0	0	0	0	0	0	0	Anticomplementary.
4557	Jan. 4	D	Jan. 8	---do----	0.1	1.0	+	tr	0	+	tr	0	Negative.
		E	Jan. 11	---do----	0.1	1.0	+	tr	0	+	tr	0	Do.
		F	Jan. 14	---do----	0.1	1.0	+	±	0	+	±	0	Do.
		G	Jan. 16	---do----	0.1	1.0	+	+	0	+	+	0	Do.
		A	Jan. 5	Jan. 5	0.1	1.0	+	+	tr	+	+	tr	Do.
		B	---do----	Jan. 16	0.1	1.0	+	+	0	+	+	0	Do.
		C	---do----	---do----	0.1	1.0	±	0	0	±	0	0	Do.
4558	---do----	D	Jan. 8	---do----	0.1	1.0	+	0	0	+	0	0	Do.
		E	Jan. 11	---do----	0.1	1.0	+	tr	0	+	tr	0	Do.
		F	Jan. 14	---do----	0.1	1.0	+	±	0	+	±	0	Do.
		G	Jan. 16	---do----	0.1	1.0	+	+	0	+	+	0	Do.
		A	Jan. 5	Jan. 5	0.1	1.0	+	+	tr	+	+	tr	Do.
		B	---do----	Jan. 16	0.1	1.0	+	+	0	+	+	0	Do.
		C	---do----	---do----	0.1	1.0	tr	0	0	tr	0	0	Do.
4559	---do----	D	Jan. 8	---do----	0.1	1.0	+	0	0	+	0	0	Do.
		E	Jan. 11	---do----	0.1	1.0	+	tr	0	+	tr	0	Do.
		F	Jan. 14	---do----	0.1	1.0	+	±	0	+	±	0	Do.
		G	Jan. 16	---do----	0.1	1.0	+	0	0	+	0	0	Do.
		A	Jan. 5	Jan. 5	0.1	1.0	+	±	0	+	+	tr	Moderately positive.
		B	---do----	Jan. 16	0.1	1.0	+	0	0	+	+	0	Strongly positive.
4560	---do----	C	---do----	---do----	0.1	1.0	0	0	0	+	0	0	Do.
		D	Jan. 8	---do----	0.1	1.0	0	0	0	+	0	0	Do.
		E	Jan. 11	---do----	0.1	1.0	0	0	0	+	0	0	Do.
		F	Jan. 14	---do----	0.1	1.0	0	0	0	+	0	0	Do.
		G	Jan. 16	---do----	0.1	1.0	0	0	0	+	0	0	Do.
		A	Jan. 5	Jan. 5	0.1	1.0	tr	0	0	+	+	0	Do.
		B	---do----	Jan. 16	0.1	1.0	0	0	0	+	+	0	Do.
		C	---do----	---do----	0.1	1.0	0	0	0	+	0	0	Do.
4561	---do----	D	Jan. 8	---do----	0.1	1.0	0	0	0	+	±	0	Do.
		E	Jan. 11	---do----	0.1	1.0	0	0	0	+	+	0	Do.
		F	Jan. 14	---do----	0.1	1.0	0	0	0	+	+	0	Do.
		G	Jan. 16	---do----	0.1	1.0	0	0	0	+	+	0	Do.

Table IX shows that fresh sera heated to 55° C. for thirty minutes before they were mixed with glycerin did not become anticomplementary in eleven days, while fresh sera that were heated after they had been mixed with glycerin were anticomplementary eleven days later. Two, Nos. 4559 and 4560, of the five sera tested had become permanently anticomplementary in eleven days; the anticomplementary property was but partially destroyed by heating portions G to 55° C. for thirty minutes. The sera were sterile.

TEST 9′

Anticomplementary serum tested.—Portions C of sera 4557, 4558, 4559, 4560, and 4561 were retested. Instead of 1 unit of amboceptor, 1.5 units were used.

TABLE IX′.—*Moderately anticomplementary sera tested by the Wassermann method.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Serum.	Amboceptor.	Tube—						Result.
							1	2	3	1′	2′	3′	
	1916.		1916.	1916.	*cc.*	*Unit.*							
4557	Jan. 4	C	Jan. 5	Jan. 16	0.1	1.5	+	±	0	+	±	0	Negative.
4558	...do....	C	...do....	...do....	0.1	1.5	+	+	0	+	+	0	Do.
4559	...do....	C	...do....	...do....	0.1	1.5	+	±	0	+	±	0	Do.
4560	...do....	C	...do....	...do....	0.1	1.5	+	0	0	+	+	0	Strongly positive.
4561	...do....	C	...do....	...do....	0.1	1.5	0	0	0	+	+	0	Do.

Table IX′ shows that moderate anticomplementary property was readily overcome by increasing the quantity of hæmolytic amboceptor.

TEST 10

The anticomplementary property of glycerin.—Specimens 4595, 4596, 4597, 4598, and 4599 were secured on February 10, 1916. On February 11, 1916, the sera were drawn off the clots. Each serum was divided into two portions, A and B. Both portions were heated to 55° C. for thirty minutes. Portion A was tested by the Wassermann method without having been mixed with glycerin. Portion B was mixed with an equal volume of sterilized, chemically pure glycerin and was tested by the Wassermann method.

TABLE X.—*The anticomplementary property of glycerin mixed with serum.*

No. of serum.	Date secured.	Portion.	Date heated.	Date tested.	Serum.	Amboceptor.	Tube—						Result.
							1	2	3	1′	2′	3′	
	1916.		1916.	1916.	*cc.*	*Unit.*							
4595	Feb. 10	A	Feb. 11	Feb. 11	0.1	1.2	+	+	±	+	+	±	Negative.
		B	...do....	...do....	0.1	1.2	+	+	0	+	+	0	Do.
4596	...do....	A	...do....	...do....	0.1	1.2	0	0	0	+	+	±	Strongly positive.
		B	...do....	...do....	0.1	1.2	0	0	0	+	+	0	Do.
4597	...do....	A	...do....	...do....	0.1	1.2	+	±	±	+	±	±	Negative.
		B	...do....	...do....	0.1	1.2	+	+	0	+	+	0	Do.
4598	...do....	A	...do....	...do....	0.1	1.2	+	0	0	+	+	±	Strongly positive.
		B	...do....	...do....	0.1	1.2	0	0	0	+	+	0	Do.
4599	...do....	A	...do....	...do....	0.1	1.2	+	tr	0	+	+	±	Do.
		B	...do....	...do....	0.1	1.2	+	0	0	+	+	0	Do.

Table X shows that the serum mixed with glycerin was slightly anticomplementary. This anticomplementary property did not affect the result obtained with the test.

CONCLUSIONS

Formalin is not a suitable preservative for serum intended for the Wassermann reaction. Sera that gave moderately positive results before formalin was added gave negative results, or nearly negative results, a week after the formalin had been added.

Glycerin kept the sera sterile and did not noticeably influence the Wassermann reaction nor the Tschernogubow modification of the Wassermann reaction.

Unheated, old sera were strongly anticomplementary. Fresh sera that were heated to 55° C. for thirty minutes before they were mixed with glycerin did not become anticomplementary in eleven days.

Fresh sera that were heated to 55° C. after they had been mixed with glycerin were anticomplementary on the eleventh day after the heating.

Nearly all unheated sera that were mixed with glycerin and were kept at room temperature became permanently anticomplementary. Anticomplementary sera could be tested provided the amboceptor was increased.

The anticomplementary property did not alter the result obtained with the Wassermann reaction or with the Tschernogubow modification of the Wassermann reaction.

A mixture of equal parts of glycerin and serum was slightly anticomplementary as compared with the serum alone.

140085——2

A CASE OF INFESTATION WITH DIPYLIDIUM CANINUM [1]

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THREE TEXT FIGURES

It has been my good fortune to find at the autopsy table four chains composed of melon-seedlike tapeworm segments in the small intestine of a male child.

A brief summary of the history is as follows:

José Abad, 8 months old, born in Pila, Laguna Province, residing at Binondo, Manila, was admitted in the department of pediatrics, Philippine General Hospital, on August 21, 1915, on account of enlargement of the abdomen. He is the seventh child of the family and the delivery was normal, although the child had a rather prominent abdomen and was entirely breast-fed.

The present illness has been present since birth. The abdomen was always distended and tympanitic and always retained the enlarged size. The mother, to relieve her child, used to give him an enema of some herb decoction, and after the expulsion of the fæcal matter and a good amount of gas, the abdomen would decrease in size and become soft. The child has always been constipated and did not thrive well, although the mother had sufficient milk.

The physical examination showed an underdeveloped and poorly nourished infant with open anterior fontanelle, large mouth due to the habit of sucking the fingers and hands, and large, distended tympanitic abdomen without muscular rigidity or spasm and showing the coils of the large intestine. The peristaltic waves could not be induced by application of cold. No one in the family has the same trouble as this boy.

Stool examination: Negative.

Urine examination: Reaction, acid; sugar, negative; albumen, a decided trace. The microscopic examination showed numerous crystals of acid sodium phosphate, epithelial cells, and some mucus.

TABLE I.—*Examination of the blood.*

Hæmoglobin	80 per cent.
Leucocytes	8,875
Polynuclears	76 per cent.
Small lymphocytes	23 per cent.
Transitionals	1 per cent.

[1] Received for publication January 18, 1916.

The clinical diagnosis was Hirschsprung's disease (megacolon), hydrocele, umbilical hernia, and rachitis.

My report of the autopsy in full, performed on September 10, 1915, is as follows:

The body is that of an underdeveloped and greatly emaciated Filipino male child, measuring 58 centimeters in length and weighing 3.46 kilograms. Rigor mortis and hypostasis are not present. The abdomen is so much dilated and the extremities so small that the body looks like that of a frog. The anterior fontanelle is open, but the sutures are closed. The face has an expression of suffering. The eyelids are open. The pupils cannot be distinguished. The lips are reddened as well as the tongue and buccal mucosa. The thorax is well expanded, but the muscles are poorly developed; the intercostal spaces are depressed, and the costochondral joints are marked. The abdominal wall is very thin and is uniformly distended. In the right lower quadrant there is an egg-shaped mass which is well formed but can be pressed and made to assume different shapes. The coils of the intestines are well marked. On turning the baby to one side, the distention is still present. The inguinal glands are not very much enlarged. The rectum admits the small finger very well.

On section subcutaneous fat is absent. The musculature is very thin. The peritoneum is pinkish, and there are about 20 cubic centimeters of sticky fluid in the peritoneal cavity. All the coils of the intestines are dilated, but the large colon is enormously hypertrophied and dilated. The appendix measures 5 centimeters in length, is under the mesentery of the ileocæcal valve, and contains some fæces. The ascending colon has a slight twist upon its longitudinal axis; it is bluish and large, having a diameter of approximately 3 centimeters. The transverse colon is very much dilated and measures 6 centimeters in diameter. The descending colon measures 4 centimeters in diameter, and the upper part of the sigmoid flexure is about 3 centimeters in diameter, curves toward the right side in a twisted way and is well covered by the peritoneum and by the other portions of the large intestine. The sigmoid and rectum are just as long as the ascending, transverse, and descending parts of the colon. The rectum measures approximately 10 to 12 centimeters in diameter and contains large fæceslike masses; it diminishes in diameter toward the anus. The inferior portion of the rectum has a diameter of 2 centimeters. The walls of the large intestine are very thick and measure about 9 millimeters in thickness. The mesentery of the large intestine has a lower origin than that of the small intestine, and the longest place of the mesentery of the large intestine (or mesosigmoid) measures 11 centimeters. All the blood vessels and lymph channels are very prominent, as well as the mesenteric glands. The small intestine has a thin and congested wall, and nearer the stomach the diameter becomes normal. The stomach is not markedly dilated and is covered by the transverse colon. The liver is small, and the diaphragm reaches the second space on the right side. The omentum is almost absent, and what remains seems to be mucoid tissue.

Thorax.—The thymus gland is very small (0.74 gram) and is pinkish yellow.

Heart.—The pericardium contains about 3 cubic centimeters of clear, straw-colored, viscid fluid. The heart is dark blue and is small. Foramen ovale is patent, and the orifices of the heart are apparently normal.

The musculature of the heart is opaque, swollen, and dark blue, and the striæ are not visible. Heart weighs 19 grams.

Lungs.—The lungs are somewhat smaller than normal, although both crepitate fairly well and float in water; they are pinkish, except their posterior portions, which are dark blue. Both lungs cut easily, and the cut surfaces show a spongelike pinkish substance. The bronchi are free from mucus, and the bronchial glands are not enlarged.

The spleen is small, and fœtal lobulations are present. It is firm and cuts with slight resistance. The cut surface is dark blue, and the splenic pulp can be easily scraped away. Malpighian bodies and trabeculæ are not very prominent. The spleen weighs 12 grams.

The adrenals are small and flat. They are firm, brownish yellow, and cut easily; the cortex is firm and is dark yellow. The two adrenals weigh 2.7 grams.

The kidneys are small, firm, and dark blue. The cut surface exudes very much blood; it is dark purple. The cortex is small and looks swollen and glistening; the pyramids are very dark. The Malpighian bodies are not visible. The two kidneys weigh 37 grams.

The liver is small, as already mentioned, and is dark purple; the edges are rounded. It is somewhat firm and cuts with slight resistance. The cut surface exudes much blood, shows a dark purplish color, is glistening, and looks oily. The liver weighs 144 grams.

The stomach contains some fluid with white flocculi. No erosion is found in it. The pyloric opening has a circumference of 2 centimeters. The duodenum is not well tinged with bile.

The gall bladder contains thin golden bile, and its ducts are patent.

The pancreas is reddish pink and does not show any marking.

Intestines.—The small intestine contains a reddish mucoid material; it has a thin wall. Toward the upper part of the ileum four chains of tapeworms were found. In the lower part of the intestine was found an isolated segment which seems to be composed of two parts that are united. The superior part enlarges and swells, and later on a small protrusion appears in the middle of it, which elongates and enlarges also, and in this way the segment creeps forward and from place to place. The posterior part does not take any part in this creeping; only it becomes smaller and shorter when the upper part enlarges. Under the microscope this segment shows a rather movable anterior part, but it is not distinctly separated from the rest. The tapeworms that are found have very small bodies and seem to be very young. Examination of the fæces (2 slides) does not show any eggs. The mucosa of the lower part of this intestine is paler and more desquamated than the upper part. The ileocæcal valve is patent, and Peyer's patches above this are all swollen and congested. The large intestine contains grayish, pasty, and sandlike material, and large pieces of this are impacted around small, hard fæces. This material seems to be bismuth. On cleaning the colon, the mucosa is found to be reddened, and in some places minute holes are seen which seem to involve only the mucosa. In the lower part of the rectum there is a large, round, necrotic area, which is approximately 2 centimeters in diameter. Inside this there are some erosions, the bases of which show white material. This is placed opposite the mesenteric attachment. A few centimeters above this there are, also, two longitudinal red ulcers that are transverse to the long axis of the colon. No stenosis nor intussusception can be found in the

large intestine, except that the intestinal wall, especially the muscular layer, looks very thick with prominent and dilated vessels and the lower part contains these ulcers which seem to be very recent. (Patient had no bowel movements twenty-four hours before death, and a Hegar's dilator was introduced into the rectum.)

The prostate gland is apparently normal.

The urinary bladder is apparently normal.

Throat organs.—The cervical glands are reddened and slightly enlarged. The thyroid gland is small; otherwise it is apparently normal.

Brain.—The cerebrospinal fluid is somewhat increased in amount, and the meninges of the brain are congested; otherwise it is apparently normal.

Anatomical diagnosis.—Dilatation and hypertrophy of the colon (megacolon); teniasis (*Dipylidium caninum*); enteritis, catarrhal; ulcerative colitis; passive congestion of the visceral organs; parenchymatous degeneration of the heart, liver, and kidneys; extreme emaciation; rickets; fœtal lobulations of the spleen; bismuth impaction in the large intestine; lymphadenitis, mesenteric and cervical; bismuth poisoning (?).

IDENTIFICATION OF THE PARASITE

I placed the reddish yellow flat segment on a slide and took it to Mr. L. D. Wharton, instructor in zoölogy, University of the Philippines, who, after seeing the chains, identified them as those of *Dipylidium caninum* and the segment as a proglottid

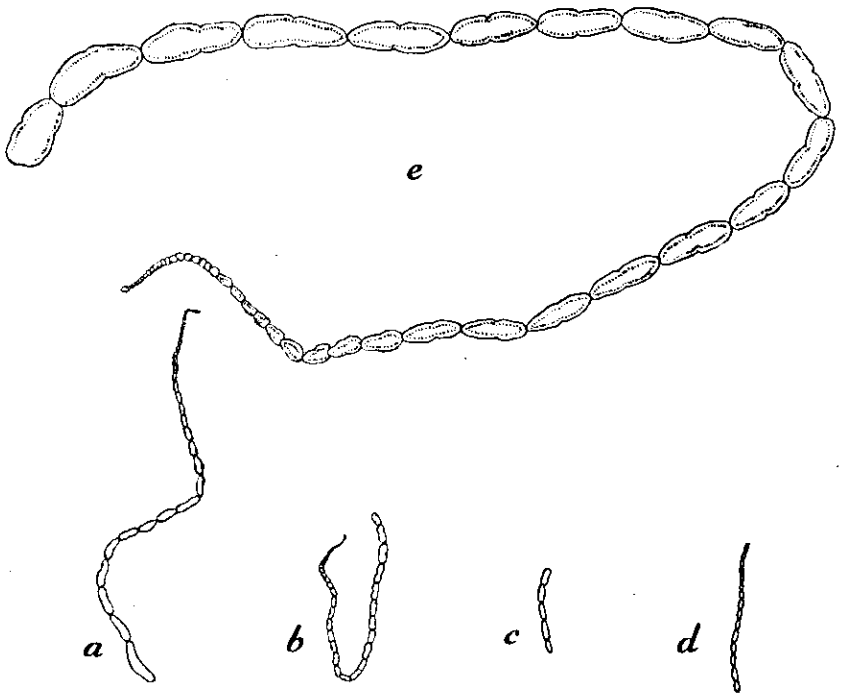

FIG. 1. *Dipylidium caninum.* *a* and *b*, natural size of worm; *c* and *d*, natural size of segments; *e*, the worm enlarged. × 5.

of the same species. Later he kindly showed me some of the internal structures of the segments of this parasite.

The segments, as mentioned, look like seeds of a melon with the pointed end of one sticking a little into the rounded end of the segment in front of it (fig. 1). Each one of the large, flat segments has about the middle a shallow groove on either side. The size of each of the segments diminishes toward the head and becomes smaller after staining.

Only one of the parasites has the neck and head, which was buried in the mucosa of the small intestine, so that I had to clip this to preserve the head. This chain has about forty segments and measures after staining 48 millimeters. The head is small, is rhomboid in shape, and under $\frac{1}{6}$ power and No. 4 objective of a C. Zeiss microscope, it shows the retracted rostellum with the four rows of alternating, rose-thornlike hooklets and two unarmed and somewhat elliptical suckers which hide the other two (fig. 2).

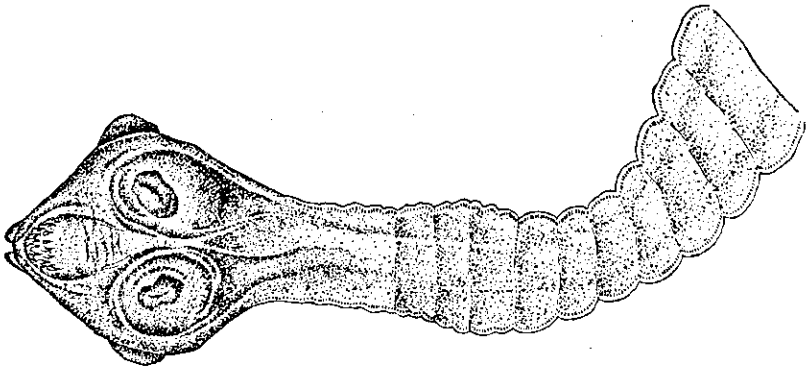

FIG. 2. Head and anterior segments of *Dipylidium caninum*.

The neck is short and does not show marked segmentation. The largest segment is 3 millimeters in length and 1 millimeter in breadth after staining.

The mature segments (fig. 3) have a slight convex outline and show a bilateral arrangement of the reproductive system. The genital pores are double and open about the middle of the segments and opposite each other. The ovaries are bilobed. The vitelline glands lie posterior to the ovaries. The vagina is posterior to the cirrus pouch. The testicles are numerous and are found in the meshes of the reticulum of the uterus.

The vasa deferentia are long, follow a tortuous course, and enter the cirrus pouch which is long and slender.

In the gravid segments the testes and ovaries disappear and the uterus is broken up into capsules which contain the eggs.

The second chain is longer; it is composed of twenty-three segments, which are larger than those of the first. The head is missing, but a part of the neck remains. The chain measures after staining 72 millimeters, and the largest segment is 7 millimeters by 2 millimeters.

The third chain is 2 centimeters long, has no head nor neck, but one end of it is finer and has smaller segments than the other end.

The last chain is composed of five segments and measures 11 millimeters in length after staining.

These parasites are placed in the museum of the College of Medicine and Surgery, University of the Philippines (No. 1495).

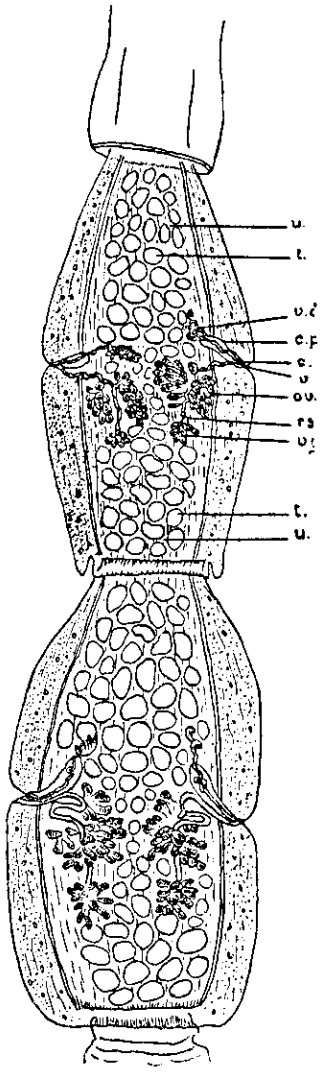

FIG. 3. Posterior segments of *Dipylidium caninum*. *u*, uterus; *t*, testicles; *vd*, vas deferens; *cp*, cirrus pouch; *c*, cirrus; *v*, vagina; *ov*, ovary; *rs*, receptaculum seminis; *vg*, vitelline gland.

BIOLOGY OF THE PARASITE

This worm is a parasite of carnivorous animals, accidentally of man and especially of children. It has been classified under Cestoda, family Hymenolepididæ, subfamily Dipylidinæ (1) and the description of it has been fully given by C. W. Stiles. (2)

The adults are normally found in the intestines of small dogs (*Canis familiaris*) and cats (*Felis catis*) in great number where they grow rapidly. The ripe proglottides are so active, as shown in this case, that they can pass by their own movements through the anus of the host with the fæcal material. Usually the segments are isolated and few, although a great number can come out. As soon as they are out of the anal ring, they crawl toward the hairy parts of the skin, where they deposit their eggs, which are swallowed by the dog louse (*Trichodectes canis*), or cat louse (*T. rostratis* Nitzsch), or are aspirated by the dog flea (*Ctenocephalis canis* Curtis), cat flea (*C. felis*

Bouché), or by that of man (*Pulex irritans* Linnæus). In these insects the eggs develop into cysticercoids (*Cryptoceptes trichodestes* Villot, 1882), as shown by Melnikow in the dog louse and by Grassi in the dog flea.

The dog is infested by biting the louse or flea and may pass the larvæ to man by licking. The cat licks its hair and gets infected or licks the milk that is later on given or taken by a child. This is graphically denounced by R. Blanchard.(3)

Infestation cannot take place directly, according to Riley,(4) from swallowing eggs or segments of the parasite, but only through ingestion of the intermediary host—dog louse and cat flea—as in the other species of tænia.

Man may accidentally ingest one of these insects, and the parasites are able then to complete their development in the host. Children are often infested due to their intimate relation with dogs and cats; besides, they do not pay much attention to their food.

Frequency of these parasites in the dog.—Bowman(5) and Willets(6) showed the presence of these parasites in the dogs of the Philippines. Krabbe(7) found 78 per cent of dogs and 66 per cent of cats infested in Copenhagen, and Ward(7) found it in from one fifth to four fifths of all dogs examined by various European investigators and says that it is common in Nebraska, in the United States. Wharton found *Dipylidium caninum* in 46.6 per cent of the dogs of Manila.

OCCURRENCE IN THE PHILIPPINE ISLANDS

The cestodes are not common intestinal parasites of man, and among them *Dipylidium caninum* is one of the rarest. I have not been able to find any authentic record of it in these Islands, although its probable occurrence has already been foreseen by some workers.

Bowman,(5) in April, 1910, suspected that the Igorots in Benguet subprovince might be infested with this parasite, owing to the fact that dog meat is one of their constant foods. Although he found the fleas *Pulex serraticeps* and *P. irritans* in that region, he did not see any eggs of *Dipylidium caninum* in 100 stool examinations. Nevertheless, he autopsied four dogs and found *Ankylostoma trigonocephalum* (Rud.) and *Dipylidium caninum.*

In October of the same year Willets(6) called the attention of the physicians of these Islands to the probability of human infection with this parasite, for it was common in dogs, and he gave the symptoms and description of the parasite.

The following table of the workers on intestinal parasites in the Philippine Islands will serve for easy comparison:

TABLE II.—*Workers on intestinal parasites in the Philippine Islands.*

Date.	Observer.	Persons infected.	*Tænia.*	*Dipylidium caninum.*	Locality.
1901 (8)	Strong	[a] 2,179	2	None	Manila.
1902 (9)	do	[b] 600	[d] 5		Do.
1905					
1907 (10)	Garrison		[e] 50	None	
1908 (11)	do	3,447	35	do	Bilibid Prison.
June-July, 1908 (12).	Gabriel	580	3	do	Hospicio de San Juan de Dios.
March-August, 1909.		1,089	6	do	Bilibid Prison.
		140		do	Bureau of Science.
1909 (13)	Garrison and Llamas	342	1	do	Manila.
1909 (14)	Garrison, Leynes, and Llamas.	945			Taytay, Rizal.
1910 (15)	Rissler and Gomez	5,406	4	None	Las Piñas.
1910	do	692	17	do	Santa Isabel.
1910	do	1,932	32	do	Tuguegarao.
1910 (16)	Chamberlain, Bloombergh, and Kilbourne.	110	15	[f]	Baguio.
July 1, 1907 (16). March 3, 1910.	do	183	9	None	Civil Hospital Baguio.
1910 (5)	Bowman	100	2	do	Baguio.
1911 (17)	Willets	3,656	59	do	Cagayan Valley.
1911 (18)	Stitt	932	3	do	Cavite.
1913 (19)	Willets	400	1	do	Batanes Islands.
1913 (20)	Crowell and Hammack	[c] 500	1	do	College of Medicine and Surgery.
1913 (21)	Tenney	250	1	do	Philippine Scouts.

[a] Stools, 1,793; autopsies, 386.
[b] Stool examinations.
[c] Autopsies.
[d] Species.
[e] Three genera and 5 species.
[f] One egg supposed to be of *Dipylidium caninum*, but no confirmatory evidence.

Dr. J. D. Jungmann tells me that during his two years' experience in the clinical laboratory of the Philippine General Hospital he has not met with the eggs or segments of this parasite.

Human infestation with *Tænia* is very rare in the Orient, and none of the reports that I could find records this parasite.

TABLE III.—*Showing freedom of man from Tænia and Dipylidium infestation in the Orient.*

Observer.	Stool examinations.	*Tænia.*	*Dipylidium caninum.*	Locality.
C. P. Kindleberger (22)	7,768	None	None	Guam.
Do	8,691	do	do	
T. H. Johnston (23)		do	do	Queensland.
F. Lindsay Woods (24)		do	do	Southern China.
J. Bell (25)	850	do	do	Hongkong.
Alfred Reed (26)	120	do	do	Changsha, China.

In the annual report of the Sanitary Commissioner with the Government of India for 1913 (Calcutta) *Bothriocephalus latus* is recorded in 18 cases, *Tænia asiatica* in 4, *T. saginata* in 21, and *T. solium* in 288, but no case of *Dipylidium* is recorded. Reports from different places in China, published in the China Medical Journal, indicate that *Tænia* infestation is rare and that *Dipylidium caninum* is conspicuous by its absence.

In the article "Medical conditions in the Torrid Zone," the data for which were collected by the College of Medicine and Surgery, University of the Philippines, in 1912, the tapeworm was not found in American Samoa nor in Korea, and 99 per cent of the intestinal worms in China were chiefly *Ascaris*.

In Formosa J. P. Maxwell found only one case of *T. solium* among 15,000 patients.(27)

In the United States of America we have the classical report and description of C. W. Stiles(2) of a child 16 months old at Detroit, Michigan, in 1903, and that of W. A. Riley(4) in 1910 of a boy 11 years old, who was very fond of a bull terrier which was later found to be infested with *Dipylidium caninum*.

In looking for new cases during recent years, the following reports were interesting. Wood(28) reported the intestinal parasites from the different laboratories in the southern part of the United States and found 10 cases of *T. saginata* and 1,004 of *Hymenolepis nana* in 1912.

In 1914 Judkins,(29) from 15,000 stool examinations in Texas, found 71 cases of *Hymenolepis nana*, 67 of *T. saginata*, and 2 of *T. cucumerina*. Of the last he states that they are rare and have no clinical interest, except that children are probably infected from fondling and kissing cats and dogs.

In order to shorten this report, let me quote the various memoirs of Blanchard,(30) before the Academy of Medicine in Paris, 1913, who collected 76 authentic cases and tabulated them according to their geographical distribution.

TABLE IV.—*Geographical distribution of cases of Dipylidium caninum infestation.*

	Cases.		Cases.
France	6	Holland	1
Germany	16	Italy	2
Austria	10	Norway	1
Cape Colony	1	Russia	3
Denmark	21	Sweden	2
United States	2	Switzerland	7
England	3	Venezuela	1

He states that he was unable to find the original papers of a case of Weinland, cited by Monti, of a 6-month-old child,

who expelled the worm with the head spontaneously, and that of Stitt, cited by Stiles and Hassel. I have been especially interested in the latter, for it might refer to the Philippines, but unfortunately I have been also unable to find the original paper.

In Blanchard's collection *Dipylidium caninum* has been found in all ages, but more frequently in children.

TABLE V.—*Blanchard's collection of human Dipylidium caninum infestation.*

Age.	1907	1914	Total.
5 weeks to 6 months	20	4	24
7 to 12 months	7	1	8
13 to 24 months	9	1	10
2 to 3 years	2	1	3
9 months to 3 years	7	0	7
4 to 8 years	6	6	12
9 to 20 years	2	2	4
Above 20 years	6	2	8
	59	17	76

PATHOLOGY

Tapeworms have to fight for the preservation of their species and their lives. To accomplish the first, they are endowed with an enormous capacity to lay eggs. To defend themselves against the action of the digestive juices, they elaborate and excrete an antibody—an antitrypsin (Weinland and Haniel);(31) or an antikinase (Dastre and Stessano) (31)—a body comparable with that elaborated by the cells of the intestinal mucosa.

That they are also able to secrete offensive substances, Flury (32) demonstrated in the case of *Ascaris*, and Blanchard (3) states that the burning and itching of the anus during the exit of this worm is due to a toxic substance secreted by this parasite, and that part of the reflex phenomena and especially those disturbances of nutrition as arrest of growth, loss of appetite, and weakness are due to the toxic substance. According to Adami (31) these toxic subtances diffuse into the tissues and into the blood and bone marrow and other seats of origin of eosinophile cells and stimulate their proliferation and increased production.

In view of the above facts one is tempted to think that, taking for granted that the infestation of my case took place soon after birth, owing to his habit of sucking his fingers, which had been probably licked by a cat or dog, and the exit of the parasite producing much discomfort, chronic constipation and final hypertrophy and dilatation of the colon occurred.

But not all cases of megacolon were found infested with *Dipylidium caninum* as shown by Finney's paper,(33) nor have these been found at the autopsies with greater frequence.

As to the lesions they produce, Blanchard(3) speaks of the suctorial action of the suckers and cites Schefferdecker who found in some dogs, that had the parasites for a long time, a considerable hypertrophy of the villi, which were from four to five times larger than normal with a rich network of capillaries, and in others the mucosa had true tunnels running longitudinally with two or three worms inside.

REASONS FOR ITS INFREQUENCE IN THE PHILIPPINE ISLANDS

The fact that this parasite has been found in dogs (Willets, Bowman, Wharton) and yet not found in man with the frequence that was expected in these Islands is due, I think, to the following reasons:

1. Filipino children, especially those of the lower classes, are as a rule not fond of playing with dogs and cats.
2. Puppies and cats are not usually given milk, but soft boiled rice, in a separate bowl when the inhabitants of the house have finished their meals.
3. Babies are almost always breast-fed, and if given artificial feeding during the later years of infancy they are under the prescription of a physician to avoid the so-called infantile beriberi.
4. Babies in the small huts of the small towns are not left sleeping on the floor, but are placed in hammacks. They are thus little in contact with cats and dogs.
5. Although some tribes eat dog meat, they burn off the hair before removing the skin.

PROPHYLAXIS

Prophylaxis is brought about by removing the cats and dogs or freeing them from their intestinal and external parasites. Those who cannot afford to buy cribs should continue the custom of placing the babies in native hammacks, as I suggested before(34) and must teach their children not to put their fingers, or anything that they find, into their mouths, as well as not to play with cats and dogs.

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ILLUSTRATIONS

[Drawings by J. Castro.]

TEXT FIGURES

FIG. 1. *Dipylidium caninum.* *a* and *b*, natural size of worm; *c* and *d*, natural size of segments; *e*, the worm enlarged × 5.

2. Head and anterior segments of *Dipylidium caninum.* (Zeiss No. 4 objective and ⅓ occular.)

3. Posterior segments of *Dipylidium caninum.* *u*, uterus; *t*, testicles; *vd*, vas deferens; *cp*, cirrus pouch; *c*, cirrus; *v*, vagina; *ov*, ovary, *rs*, receptaculum seminis; *vg*, vitelline gland. (Zeiss No. 4 objective and ⅓ occular.)

HÆMOLYSIS BY HUMAN SERUM [1]

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Many different corpuscles have been advocated for the complement-fixation test for syphilis, utilizing the human complement and hæmolytic amboceptor normally present in the serum to be tested, and many different hæmolytic systems have been and still are being employed in the Wassermann reaction and modifications thereof.

For methods that make use of the natural human complement and hæmolytic amboceptor, Hecht [2] used the corpuscles of the sheep. Tschernogubow first (cited by himself) advocated the use of sheep corpuscles. Later he [3] recommended the corpuscles of the guinea pig. Gurd [4] advocates the use of guinea pig corpuscles. The corpuscles of the hen and of other animals have been suggested by other writers.

In conducting the complement-fixation test using alien complement and hæmolytic amboceptor, the sheep hæmolytic system is commonly employed with human serum. The sheep hæmolytic system is not an ideal one to be used with human serum because of the presence of natural antisheep hæmolytic amboceptor in human serum that is readily reactivated by guinea pig complement. Bauer [5] used guinea pig complement, but utilized the antisheep hæmolytic amboceptor normally present in human serum. Noguchi [6] emphatically states that human corpuscles and a corresponding amboceptor must be used in order to get reliable results.

The human hæmolytic system is an ideal one because isohæmolysins practically never exist. The antihuman hæmolytic system is not entirely without faults; animals rarely if ever produce a highly potent antihuman hæmolytic serum, and frequently it is difficult to obtain suitable corpuscles for conducting the tests.

[1] Read before a meeting of the Manila Medical Society, February, 1916. Received for publication October 21, 1915.

[2] *Wien. klin. Wochenschr.* (1908), 21, (1909), 22, 265; (1909), 22, 338.

[3] *Deut. med. Wochenschr.* (1909), 35, 668.

[4] *Journ. Infect. Dis.* (1911), 8, 427.

[5] *Deut. med. Wochenschr.* (1909), 35, 432.

[6] *Journ. Exp. Med.* (1909), 11, 392; *Münch. med. Wochenschr.* (1909), 56, 494.

While the literature contains many references to the presence of natural antisheep hæmolysin in human serum, very little has been done toward overcoming the difficulty by finding a more suitable hæmolytic system. Sachs [7] gives a summary of the work done, and more recently Kolmer and Casselman [8] have more extensively reported on the presence in human serum of hæmolysins for the corpuscles of sheep, dog, ox, goat, hog, rat, chicken, horse, rabbit, and guinea pig. According to the findings of these authors, the hog, rat, chicken, horse, rabbit, or guinea pig hæmolytic systems are to be preferred to the sheep hæmolytic system.

In the following report are recorded the results obtained by testing fifty unheated human sera for hæmolysins against the corpuscles of the sheep, goat, horse, rabbit, and guinea pig and the results obtained by testing fifty reactivated, heated human sera for hæmolysins against the corpuscles of the same animals.

HÆMOLYSIS BY UNHEATED HUMAN SERUM

Technique.—Fifty human sera were tested within twenty-four hours of the bleeding in the following quantities: 0.4, 0.2, 0.1, 0.05, and 0.025 cubic centimeter. The results obtained with 0.025 cubic centimeters are omitted from the tables because only six sera produced a trace of hæmolysis, while all others gave negative results. To each test tube with serum, enough physiologic salt solution (0.9 per cent) was added to make 1.5 cubic centimeters; corpuscles were added in quantity of 1 cubic centimeter of a 2 per cent suspension, which brought the total quantity in each test tube up to 2.5 cubic centimeters. After each tube had been shaken, it was placed in the incubator at about 37° C. for one hour. During this hour each test tube was shaken at least four times. After an hour in the incubator the tubes were allowed to stand at room temperature (25° C. to 30° C.) and the results were read and recorded about three hours after the corpuscles had been added.

Hæmolysis of sheep corpuscles.—Table I shows the results obtained with sheep corpuscles. Sheep-blood corpuscles are readily dissolved by fresh, unheated human serum. None of these fifty human sera failed completely to dissolve the test dose of corpuscles when 0.4 cubic centimeter of serum was used.

[7] Kolle und Wassermann, Handbuch der pathogenen Microorganismen (1913), 2, 799.

[8] *Journ. Infect. Dis.* (1915), 16, 441.

TABLE I.—*The effect of unheated human serum on sheep corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				*Per cent.*
0.4	50	0	0	0
0.2	49	1	0	0
0.1	45	3	2	0
0.05	19	10	8	26

Hæmolysis of goat corpuscles.—Table II shows that human serum has fairly good hæmolytic power for the corpuscles of the goat, but not so good as for sheep corpuscles.

TABLE II.—*The effect of human serum on goat corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				*Per cent.*
0.4	50	0	0	0
0.2	45	3	2	0
0.1	8	15	15	24
0.05	0	0	2	96

Hæmolysis of horse corpuscles.—Table III shows that the hæmolytic powers of these fifty unheated human sera for the corpuscles of the horse were approximately equal to those for the corpuscles of the goat.

TABLE III.—*The effect of unheated human serum on horse corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				*Per cent.*
0.4	49	0	0	2
0.2	44	3	1	4
0.1	20	10	11	18
0.05	1	2	6	82

Hæmolysis of rabbit corpuscles.—The hæmolytic power of unheated human serum for rabbit corpuscles was found to be much less than for the corpuscles of the sheep, goat, or horse.

TABLE IV.—*The effect of unheated human serum on rabbit corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				*Per cent.*
0.4	50	0	0	0
0.2	22	11	12	10
0.1	0	0	1	98
0.05	0	0	0	100

Hæmolysis of guinea pig corpuscles.—As is shown in Table V, unheated human serum dissolves the corpuscles of the guinea pig almost as readily as it dissolves the corpuscles of the sheep.

TABLE V.—*The effect of unheated human serum on guinea pig corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				*Per cent.*
0.4	50	0	0	0
0.2	47	2	1	0
0.1	19	15	13	6
0.05	1	3	5	82

Details of results obtained with unheated human serum.—A detailed record of the results obtained is given in Table VI.

TABLE VI.—*Hæmolysis by unheated human serum.*

[Numbers represent cubic centimeter of human serum.]

Serum No.	Corpuscles of—																			
	Sheep.				Goat.				Horse.				Rabbit.				Guinea pig.			
	0.4	0.2	0.1	0.05	0.4	0.2	0.1	0.05	0.4	0.2	0.1	0.05	0.4	0.2	0.1	0.05	0.4	0.2	0.1	0.05
1	+	+	+	+	+	+	±	0	+	+	±	0	+	+	0	0	+	+	+	0
2	+	+	+	+	+	+	+	0	+	+	+	tr	+	+	0	0	+	+	+	0
3	+	+	+	+	+	+	±	0	+	+	+	0	+	+	0	0	+	+	tr	0
4	+	+	+	+	+	+	±	0	+	+	±	0	+	±	0	0	+	+	+	0
5	+	+	+	+	+	+	±	0	+	+	+	0	+	+	0	0	+	+	+	0
6	+	+	+	±	+	+	±	0	+	+	±	0	+	±	0	0	+	+	+	tr
7	+	+	+	tr	+	+	tr	0	+	+	tr	0	+	±	0	0	+	+	±	0
8	+	+	±	0	+	+	tr	0	+	+	tr	0	+	+	0	0	+	+	±	0
9	+	+	+	tr	+	+	±	0	+	+	+	0	+	+	0	0	+	+	±	0
10	+	+	+	±	+	+	±	0	+	+	±	0	+	+	0	0	+	+	±	0
11	+	+	+	±	+	+	±	0	+	+	+	0	+	+	0	0	+	+	+	0
12	+	+	±	0	+	+	0	0	+	±	0	0	+	tr	0	0	+	+	tr	0
13	+	+	+	±	+	+	+	0	+	+	+	tr	+	+	tr	0	+	+	±	0

TABLE VI.—*Hæmolysis by unheated human serum*—Continued.

Serum No.	Corpuscles of—																			
	Sheep.				Goat.				Horse.				Rabbit.				Guinea pig.			
	0.4	0.2	0.1	0.05	0.4	0.2	0.1	0.05	0.4	0.2	0.1	0.05	0.4	0.2	0.1	0.05	0.4	0.2	0.1	0.05
14	+	+	+	±	+	+	tr	0	+	+	±	0	+	+	0	0	+	+	+	0
15	+	+	+	0	+	+	0	0	+	+	0	0	+	tr	0	0	+	+	tr	0
16	+	+	+	tr	+	+	tr	0	+	±	0	0	+	+	0	0	+	+	tr	0
17	+	+	+	0	+	+	0	0	+	+	tr	0	+	0	0	0	+	+	tr	0
18	+	+	+	±	+	+	tr	0	+	+	+	0	+	+	0	0	+	+	±	0
19	+	+	+	+	+	+	+	0	+	+	+	tr	+	+	0	0	+	+	+	+
20	+	+	+	+	+	+	±	0	+	+	+	tr	+	+	0	0	+	+	+	tr
21	+	+	+	±	+	+	tr	0	+	+	+	0	+	0	0	0	+	+	±	0
22	+	+	+	+	+	+	±	0	+	+	±	0	+	tr	0	0	+	+	±	0
23	+	+	+	0	+	+	0	0	+	+	tr	0	+	+	0	0	+	+	tr	0
24	+	+	+	+	+	+	±	0	+	+	+	0	+	+	0	0	+	+	+	tr
25	+	+	+	+	+	+	+	0	+	+	+	±	+	+	0	0	+	+	+	±
26	+	+	+	tr	+	tr	0	0	+	+	tr	0	+	0	0	0	+	±	0	0
27	+	+	+	+	+	+	±	0	+	+	0	0	+	0	0	0	+	+	±	0
28	+	+	+	+	+	+	tr	0	+	+	+	0	+	tr	0	0	+	+	±	0
29	+	+	tr	0	+	±	0	0	+	+	tr	0	+	±	0	0	+	+	tr	0
30	+	+	+	+	+	+	+	0	+	+	±	0	+	±	0	0	+	+	±	0
31	+	+	+	±	+	+	tr	0	+	+	tr	0	+	tr	0	0	+	±	tr	0
32	+	+	+	±	+	+	tr	0	+	+	tr	0	+	tr	0	0	+	+	tr	0
33	+	+	+	tr	+	+	tr	0	+	+	tr	0	+	±	0	0	+	+	tr	0
34	+	+	tr	0	+	+	0	0	+	+	+	0	+	tr	0	0	+	+	tr	0
35	+	±	0	0	+	tr	0	0	+	+	tr	0	+	tr	0	0	+	+	tr	0
36	+	+	+	+	+	+	+	tr	+	+	+	±	+	+	0	0	+	+	±	0
37	+	+	+	tr	+	+	tr	0	+	+	+	tr	+	tr	0	0	+	+	±	0
38	+	+	+	+	+	+	+	tr	+	+	+	tr	+	±	0	0	+	+	+	tr
39	+	+	+	tr	+	+	tr	0	+	+	±	0	+	±	0	0	+	+	±	0
40	+	+	+	+	+	+	±	0	+	+	±	0	+	+	0	0	+	+	+	±
41	+	+	+	0	+	+	tr	0	+	+	+	0	+	0	0	0	+	tr	0	0
42	+	+	+	+	+	+	+	0	+	+	+	+	+	+	0	0	+	+	+	0
43	+	+	0	0	+	+	0	0	+	+	0	0	+	tr	0	0	+	+	0	0
44	+	+	+	tr	+	+	±	0	+	+	±	0	+	±	0	0	+	+	±	0
45	+	+	±	0	+	±	0	0	+	+	tr	0	+	tr	0	0	+	+	tr	0
46	+	+	+	tr	+	+	tr	0	+	0	0	0	+	±	0	0	+	+	+	0
47	+	+	+	+	+	±	0	0	+	tr	0	0	+	+	0	0	+	+	+	±
48	+	+	+	0	+	+	0	0	0	0	0	0	+	tr	0	0	+	+	+	0
49	+	+	+	+	+	+	tr	0	+	±	0	0	+	±	0	0	+	+	+	0
50	+	+	+	±	+	+	±	0	+	+	+	0	+	+	0	0	+	+	+	tr

+=complete hæmolysis; ±=from 50 to 100 per cent hæmolysis; tr=hæmolysis less than 50 per cent; 0=no hæmolysis.

Table VI shows that sera 46, 47, 48, 49, and 50 have a low hæmolytic power for horse corpuscles. These five sera were tested at one time, and this drop in the hæmolytic power of these sera did not escape notice. Other things having been equal, the resistance of the corpuscles was at once thought of. As I had never used corpuscles of this horse before, serum 48,

which failed to dissolve the horse corpuscles, was retested against the corpuscles of the same horse and against the corpuscles of three other horses.

TABLE VII.—*Serum 48 tested against the corpuscles of four different horses.*

Corpuscles of horse—	Human serum.			
	0.4	0.2	0.1	0.05
1	0	0	0	0
2	+	tr	0	0
3	+	±	0	0
4	+	±	0	0

Table VII shows that the corpuscles of horse 1 were unusually resistant toward hæmolysis. While 0.4 cubic centimeter of serum 48 did not produce so much as a trace of hæmolysis with corpuscles of horse 1, the dose of 0.2 cubic centimeter almost completely dissolved the test dose of corpuscles from horses 3 and 4.

HÆMOLYSIS BY REACTIVATED, HEATED HUMAN SERUM

Technique.—The sera were heated to between 55°C. and 56°C. for thirty minutes, and the heated sera were used in quantities of 0.4, 0.2, and 0.1 cubic centimeter. As complement the pooled sera of five guinea pigs were used in a dose of 0.5 cubic centimeter of a 10 per cent dilution in physiologic salt solution. The natural hæmolytic amboceptor was not removed from the complement serum; it was tested for, and none was found in the test dose for the corpuscles of the sheep, the goat, and the rabbit. With horse corpuscles guinea pig serum produced a trace of hæmolysis. The washed corpuscles were used in dose of 0.5 cubic centimeter of a 4 per cent suspension in physiologic salt solution. Three test tubes were used for each test. In tube 1 were put 0.5 cubic centimeter of diluted, heated human serum representing 0.4 cubic centimeter serum and 0.1 cubic centimeter of physiologic salt solution, 0.5 cubic centimeter of 10 per cent dilution of guinea pig serum, 0.5 cubic centimeter of 4 per cent suspension of washed corpuscles, and 1 cubic centimeter of physiologic salt solution to make the total quantity in the tube 2.5 cubic centimeters. Tube 2 received 0.5 cubic centimeter of diluted serum representing 0.2 cubic centimeter of serum and 0.3 cubic centimeter of physiologic salt solution. Complement, corpuscles suspension, and physiologic salt solution were added as in tube 1.

Tube 3 received 0.5 cubic centimeter of diluted serum representing 0.1 cubic centimeter of serum and 0.4 cubic centimeter of physiologic salt solution. Complement, corpuscles, and salt solution were added as in the foregoing tubes. Each tube was shaken and placed in the incubator at about 37°C. for one hour, during which time the tubes were shaken at intervals of about fifteen minutes. After one hour in the incubator the tubes were removed to the refrigerator at about 7°C., and the results were read on the following morning.

Hæmolysis of sheep corpuscles.—Table VIII shows that reactivated, heated human serum readily dissolved the corpuscles of the sheep. All of the fifty sera examined produced some hæmolysis when used in a dose of 0.2 cubic centimeter. Of one serum 0.4 cubic centimeter failed to produce lysis, and of one serum the dose of 0.1 cubic centimeter remained inactive.

TABLE VIII.—*The effect of reactivated, heated human serum on sheep corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				*Per cent.*
0.4	39	4	6	2
0.2	40	7	3	0
0.1	40	4	5	2

Hæmolysis of goat corpuscles.—Table IX shows that reactivated human serum dissolved goat corpuscles almost as readily as it dissolved the corpuscles of the sheep.

TABLE IX.—*The effect of reactivated, heated human serum on goat corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				*Per cent.*
0.4	30	13	6	2
0.2	31	13	5	2
0.1	23	13	8	2

Hæmolysis of horse corpuscles.—The amount of hæmolysis obtained with horse corpuscles was much less than that obtained with the corpuscles of the sheep or of the goat. As is shown in Table X, the dose of 0.2 cubic centimeter of one serum completely dissolved the test dose of corpuscles, and of one serum 0.1 cubic centimeter completely dissolved the corpuscles. Al-

though complete solution of the corpuscles occurred in two instances only, all of the fifty reactivated sera produced some hæmolysis.

TABLE X.—*The effect of reactivated, heated human serum on horse corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				*Per cent.*
0.4	0	14	36	0
0.2	1	13	36	0
0.1	1	10	39	0

Hæmolysis of rabbit corpuscles.—In Table XI are shown the results obtained with rabbit corpuscles. Heated human serum reactivated with guinea pig complement has but weak hæmolytic power for the corpuscles of the rabbit.

TABLE XI.—*The effect of reactivated, heated human serum on rabbit corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				*Per cent.*
0.4	0	1	9	80
0.2	0	0	8	84
0.1	0	0	8	84

Hæmolysis of guinea pig corpuscles.—Table XII shows the effect of heated human serum reactivated with guinea pig complement on guinea pig corpuscles. The hæmolysis obtained was practically negligible. This failure to dissolve guinea pig corpuscles cannot be due to the absence of hæmolytic amboceptor, because in Table V it is shown that unheated human serum readily dissolved guinea pig corpuscles.

TABLE XII.—*The effect of reactivated, heated human serum on guinea pig corpuscles.*

Serum.	100 per cent hæmolysis.	50 to 100 per cent hæmolysis.	Less than 50 per cent hæmolysis.	No hæmolysis.
cc.				*Per cent.*
0.4	0	1	20	58
0.2	0	0	10	80
0.1	0	0	2	96

Table XIII shows the results obtained with the fifty reactivated human sera tested against the corpuscles of sheep, goat, horse, rabbit, and guinea pig. With the corpuscles of the rabbit and of the guinea pig very little hæmolysis was obtained. Although these sera were tested while still fresh, some were decidedly anticomplementary. This anticomplementary property is well shown in sera 1, 2, 5, 26, 28, 32, and 47; less hæmolysis was obtained with the larger quantities of serum than with the smaller quantities. Other sera, such as Nos. 24 and 50, show the anticomplementary property in a minor degree; the large quantities of serum produced no more hæmolysis than did the smaller quantities.

TABLE XIII.—*Hæmolysis by reactivated human serum.*

[Numbers represent cubic centimeter of human serum.]

Serum No.	Corpuscles of—														
	Sheep.			Goat.			Horse.			Rabbit.			Guinea pig.		
	0.4	0.2	0.1	0.4	0.2	0.1	0.4	0.2	0.1	0.4	0.2	0.1	0.4	0.2	0.1
1	±	+	+	±	+	+	tr	tr	tr	0	0	0	0	0	0
2	tr	+	+	±	±	±	tr	tr	tr	0	0	0	0	0	0
3	+	+	+	+	+	+	tr	tr	tr	0	0	0	0	0	0
4	+	+	+	+	+	+	tr	tr	tr	0	0	0	tr	0	0
5	tr	±	±	tr	±	±	tr	tr	tr	0	0	0	0	0	0
6	+	+	+	+	+	+	tr	±	±	0	0	0	0	0	0
7	+	+	+	+	+	+	tr	tr	tr	0	0	0	0	0	0
8	+	+	+	+	+	+	±	±	±	0	0	0	0	0	0
9	+	+	+	+	+	+	±	±	±	0	0	0	tr	0	0
10	+	+	+	+	+	+	tr	tr	tr	0	0	0	0	0	0
11	+	+	+	+	+	+	tr	tr	tr	tr	tr	0	tr	0	0
12	+	+	+	+	+	+	±	±	±	0	0	0	tr	tr	0
13	+	+	+	+	+	+	tr	tr	tr	0	0	0	tr	0	0
14	+	+	+	+	+	+	±	±	±	tr	0	0	0	0	0
15	+	+	+	+	+	+	±	±	tr	0	0	0	tr	tr	0
16	+	+	+	+	+	+	±	±	±	0	0	0	tr	0	0
17	+	+	+	+	+	+	tr	tr	tr	tr	0	0	tr	0	0
18	+	+	+	+	+	+	±	±	±	tr	tr	tr	tr	tr	0
19	+	+	+	+	+	+	tr	tr	tr	0	0	0	0	0	0
20	+	+	+	+	+	+	tr	tr	tr	0	0	0	tr	0	0
21	+	±	tr	+	+	±	tr	tr	tr	0	0	0	tr	tr	0
22	±	±	tr	+	+	±	tr	tr	tr	±	tr	tr	tr	0	0
23	+	+	+	+	+	+	±	±	tr	0	0	0	tr	tr	0
24	±	±	±	±	±	±	tr	tr	tr	0	0	0	0	0	0
25	tr	tr	0	±	±	tr	±	tr	tr	0	0	0	tr	tr	0
26	tr	±	±	tr	±	±	tr	tr	tr	0	0	0	0	0	0
27	+	+	+	±	+	+	tr	tr	tr	0	0	0	tr	tr	tr
28	±	+	+	±	±	±	tr	±	±	0	0	0	tr	0	0
29	+	+	+	±	±	±	tr	tr	tr	0	0	0	0	0	0
30	+	+	+	+	+	+	±	+	+	0	0	0	0	0	0
31	+	+	+	+	+	+	±	±	tr	0	0	tr	±	tr	tr
32	tr	±	±	±	±	±	tr	tr	tr	0	0	0	0	0	0

TABLE XIII.—*Hæmolysis by reactivated human serum*—Continued.

Serum No.	Corpuscles of—														
	Sheep.			Goat.			Horse.			Rabbit.			Guinea pig.		
	0.4	0.2	0.1	0.4	0.2	0.1	0.4	0.2	0.1	0.4	0.2	0.1	0.4	0.2	0.1
33	+	+	+	+	+	+	tr	tr	tr	0	0		0tr	0	0
34	+	+	+	+	+	+	tr	tr	tr	0	0	0	0	0	0
35	+	+	+	+	+	+	tr	tr	tr	tr	tr	tr	tr	tr	0
36	+	+	+	+	+	±	tr	tr	tr	0	0	0	0	0	0
37	+	+	+	±	tr	tr	tr	tr	tr	0	0	0	0	0	0
38	+	±	tr	0	0	0	±	tr	tr	tr	tr	tr	0	0	0
39	+	+	+	+	+	+	±	±	±	0	0	0	0	0	0
40	+	+	+	tr	tr	tr	tr	tr	tr	tr	tr	tr	0	0	0
41	+	+	+	±	±	±	tr	tr	tr	0	0	0	0	0	0
42	+	+	+	±	±	±	tr	tr	tr	tr	tr	tr	0	0	0
43	+	+	+	+	+	+	±	±	±	tr	tr	tr	0	0	0
44	+	+	+	±	±	±	tr	tr	tr	0	0	0	0	0	0
45	+	+	+	tr	tr	tr	tr	tr	tr	0	0	0	0	0	0
46	+	+	+	+	+	+	tr	tr	tr	0	0	0	tr	tr	0
47	0	tr	tr	tr	tr	tr	tr	tr	tr	0	0	0	0	0	0
48	+	+	+	+	±	tr	tr	tr	tr	0	0	0	0	0	0
49	+	+	+	±	±	tr	tr	tr	tr	0	0	0	tr	0	0
50	tr	tr	tr	tr	tr	tr	tr	tr	tr	0	0	0	0	0	0

+=complete hæmolysis (100 per cent); ±=hæmolysis between 50 and 100 per cent; tr=hæmolysis less than 50 per cent; 0=no hæmolysis.

Reactivation of natural antihorse amboceptor.—A quantity of fresh human serum was divided into three portions designated as A, B, and C. Portion A was left unheated, portion B was heated to about 55° C. for thirty minutes, and from portion C the natural antihorse amboceptor was removed. Portion C was not heated. The amboceptor was removed in the following manner: One cubic centimeter of unheated serum was diluted with 1 cubic centimeter of physiologic salt solution and was placed in crushed ice. A centrifuge tube with 2 cubic centimeters of washed horse corpuscles was also placed in the crushed ice. Having stood in the crushed ice for fifteen minutes, the diluted serum was poured into the centrifuge tube with the corpuscles; serum and corpuscles were well mixed and were left in the crushed ice. Four centrifuge tubes, each containing 2 cubic centimeters of washed horse corpuscles and 1 cubic centimeter of human serum, were prepared. Two hours after mixing the corpuscles with the serum one tube was centrifuged and the serum was pipetted off and tested for hæmolytic properties. It still contained most of the antihorse amboceptor. At the

end of three hours the second tube was centrifuged, and the serum was pipetted off and tested for hæmolytic power. It still dissolved the horse corpuscles. After five hours the third and fourth tubes were centrifuged, and the serum was pipetted off and tested for hæmolysin. Of this serum 0.4 cubic centimeter still produced a trace of hæmolysis with one half of the usual test dose of horse corpuscles, but 0.2 cubic centimeter of serum mixed with half the usual dose of corpuscles failed to produced hæmolysis.

Now an attempt was made to reactivate the heated human serum (portion B) with human complement (portion C) and with guinea pig complement freed from antihorse amboceptor.

Technique.—Three sets of test tubes were prepared. Each set contained three tubes designated as 1, 2, and 3. The first set received unheated human serum (portion A); tube 1 received 0.4 cubic centimeter of serum; tube 2 received 0.2 cubic centimeter of serum, and tube 3 received 0.1 cubic centimeter of serum. Each tube received 0.5 cubic centimeter of 4 per cent suspension of horse corpuscles and enough physiologic salt solution to make the total quantity 2.5 cubic centimeters. In set 2 the heated human serum was reactivated with human complement. Tube 1 received 0.4 cubic centimeter of complement serum, 0.5 cubic centimeter of 4 per cent suspension of horse corpuscles, and enough physiologic salt solution to make the total quantity 2.5 cubic centimeters. Tube 2 received 0.2 cubic centimeter of heated serum, 0.2 cubic centimeter of complement serum, 0.5 cubic centimeter of 4 per cent suspension of horse corpuscles, and enough physiologic salt solution to make the total quantity 2.5 cubic centimeters. Tube 3 received 0.1 cubic centimeter of heated serum, 0.1 cubic centimeter of complement serum, 0.5 cubic centimeter of 4 per cent suspension of horse corpuscles, and enough physiologic salt solution to make the total quantity 2.5 cubic centimeters.

In set 3 the heated serum was reactivated with guinea pig serum freed from natural antihorse amboceptor. Tube 1 received 0.4 cubic centimeter of heated serum and 0.4 cubic centimeter of complement serum. Tube 2 received 0.2 cubic centimeter of heated serum and 0.2 cubic centimeter of complement serum. Tube 3 received 0.1 cubic centimeter of heated serum and 0.1 cubic centimeter of complement serum. To each tube was added 0.5 cubic centimeter of 4 per cent suspension of horse corpuscles and enough physiologic salt solution to make 2.5 cubic centimeters.

TABLE XIV.—*Natural antihorse amboceptor reactivated.*

Set—	Serum.		
	0.4	0.2	0.1
1	+	+	±
2	+	+	tr
3	+	±	0

Table XIV shows that this human serum was readily reactivated with human complement; almost the entire hæmolytic power was restored. Guinea pig complement did not reactivate the serum well.

Reactivation of the antirabbit amboceptor in fresh human serum.—A quantity of human serum was divided into three portions—A, B, and C—and was tested for hæmolytic power within five hours after the bleeding. Portion A was not heated; it was tested for the original hæmolytic power. Portions B and C were heated to about 55° C. for thirty minutes and were reactivated with fresh guinea pig serum. The guinea pig serum, also, was tested for hæmolysin for rabbit corpuscles.

Technique.—For the human serum three sets of tubes, A, B, and C, were prepared. Each set contained three tubes designated as 1, 2, and 3. Tubes 1, 2, and 3 in set A received 0.4 cubic centimeter, 0.2 cubic centimeter, and 0.1 cubic centimeter of unheated human serum, respectively. To each tube 0.5 cubic centimeter of 4 per cent suspension of rabbit corpuscles was added, and the total quantity in each tube was brought up to 2.5 cubic centimeters with physiologic salt solution. In set B tube 1 received 0.4 cubic centimeter of heated human serum and 0.4 cubic centimeter of guinea pig serum. Tube 2 received 0.2 cubic centimeter of heated human serum and 0.2 cubic centimeter of guinea pig serum, while tube 3 received 0.1 cubic centimeter of heated human serum and 0.1 cubic centimeter of guinea pig serum. To each tube was added 0.5 cubic centimeter of 4 per cent suspension of rabbit corpuscles and enough physiologic salt solution to bring the total quantity up to 2.5 cubic centimeters.

Tubes 1, 2, and 3 in set C received 0.4 cubic centimeter, 0.2 cubic centimeter, and 0.1 cubic centimeter of heated human serum and 0.2 cubic centimeter, 0.1 cubic centimeter, and 0.05 cubic centimeter of guinea pig serum, respectively. Each tube received 0.5 cubic centimeter of 4 per cent suspension of rabbit corpuscles and enough salt solution to bring the total quantity

up to 2.5 cubic centimeters. All tubes were placed in the incubator at 37°C. for one hour and were then removed to room temperature. The results were read and recorded about three hours after the corpuscles had been added.

TABLE XV.—*Natural antirabbit amboceptor reactivated with guinea pig complement.*

Set—	Serum.		
	0.4	0.2	0.1
A	+	+	0
B	+	+	+
C	+	+	±

Table XV shows that the natural antirabbit amboceptor can be reactivated with guinea pig complement provided a sufficiently large dose is used. Equal parts of heated human serum and fresh guinea pig serum had more than double the hæmolytic power of the unheated human serum. Heated human serum mixed with half its volume of fresh guinea pig serum had nearly twice the hæmolytic power of the whole human serum.

Antirabbit hæmolysin in guinea pig serum.—Fresh guinea pig serum was tested in doses of 0.8, 0.4, 0.2, and 0.1 cubic centimeter against 0.5 cubic centimeter of 4 per cent suspension of rabbit corpuscles. The total quantity in each was made up to 2.5 cubic centimeters with physiologic salt solution, and the results were read about three hours after the corpuscles had been added.

TABLE XVI.—*Complement control to Table XV. Antirabbit hæmolysis in guinea pig serum.*

Guinea pig serum. cc.	Result.
0.8	±
0.4	tr
0.2	0
0.1	0

As Table XVI shows, this guinea pig serum had slight hæmolytic power for rabbit corpuscles.

Reactivation of natural antiguinea pig amboceptor.—In preliminary test it was found that the natural antiguinea pig amboceptor was not entirely absorbed from human serum in two hours; three hours sufficed, while five hours were too long. Human serum that had been in contact with guinea pig cor-

puscles for five hours failed to reactivate the amboceptor. This was probably due to the disappearance of complement.

Technique.—A quantity of fresh human serum was divided into three portions designated as A, B, and C. Portion A remained unheated, portion B was heated to 55°C. for thirty minutes, and from portion C the natural antiguinea pig amboceptor was removed in the following manner. One cubic centimeter of unheated human serum was diluted with 1 cubic centimeter of physiologic salt solution and was placed in cracked ice. For each tube with diluted serum a centrifuge tube with 3 cubic centimeters of washed guinea pig corpuscles was also placed in the cracked ice. After having stood in the cracked ice for fifteen minutes, the serum was mixed with the corpuscles in the proportion of 2 cubic centimeters of diluted serum to 3 cubic centimeters of corpuscles. These mixtures were allowed to remain in the cracked ice for three hours and were then removed and centrifuged; the clear serum was pipetted off and was used as complement. The complement was carefully tested for amboceptor; the dose of 0.8 cubic centimeter mixed with the test dose of corpuscles failed to produce hæmolysis.

Now it was attempted to reactivate the heated human serum with the human complement and with guinea pig complement.

Three sets of tubes, designated A, B, and B′, were prepared. Each set contained four tubes marked 1, 2, 3, and 4. Tubes 1, 2, 3, and 4 of set A received 0.4, 0.2, 0.1, and 0.05 cubic centimeter of serum A, respectively. To each tube was added 0.5 cubic centimeter of 4 per cent suspension of guinea pig corpuscles and enough salt solution to make the total quantity 2.5 cubic centimeters. Set B received serum B and complement C. Tubes 1, 2, 3, and 4 received 0.4, 0.2, 0.1, and 0.05 cubic centimeter of heated serum B and 0.4, 0.2, 0.1, and 0.05 cubic centimeter of complement C, respectively. Each tube received 0.5 cubic centimeter of 4 per cent suspension of guinea pig corpuscles and enough salt solution to bring the total quantity up to 2.5 cubic centimeters. In set B′ serum B and guinea pig complement were used. Tubes 1, 2, 3, and 4 received 0.4, 0.2, 0.1, and 0.05 cubic centimeter of heated serum and 0.4, 0.2, 0.1, and 0.05 cubic centimeter of fresh guinea pig serum, respectively. The usual test dose of guinea pig corpuscles was added to each tube, and the total quantity in each tube was brought up to 2.5 cubic centimeters with physiologic salt solution. After shaking, the tubes were put in the incubator at 37°C. for one hour and then removed to room temperature; the results were read about three hours after the corpuscles had been added.

TABLE XVII.—*Reactivation of natural antiguinea pig amboceptor.*

Set—	Human serum.			
	0.4	0.2	0.1	0.05
A	+	+	+	±
B	+	+	+	tr
B'	0	0	0	0

Table XVII shows the results obtained. The unheated human serum had good hæmolytic power for guinea pig corpuscles; complete hæmolysis occurred in tubes 1, 2, and 3 and more than 50 per cent hæmolysis in tube 4. Human complement added to heated human serum almost completely restored the original hæmolytic power. Guinea pig complement did not reactivate the natural antiguinea pig amboceptor.

The effect of absorption of one amboceptor on another amboceptor.—After having removed the natural antihorse amboceptor, the serum was tested for hæmolytic power against guinea pig corpuscles. Two sets of tubes, A and B, were used. Each set contained four tubes marked 1, 2, 3, and 4. In set A was tested the hæmolytic power of the whole human serum in quantities of 0.4, 0.2, 0.1, and 0.05 cubic centimeter. Each tube received 0.5 cubic centimeter of 4 per cent suspension of guinea pig corpuscles and enough physiologic salt solution to make the total quantity 2.5 cubic centimeters. In set B the human serum, freed from natural antihorse amboceptor, was titrated against guinea pig corpuscles. All tubes were shaken, placed in the incubator at 37°C. for one hour, and removed to room temperature; the results were read about three hours after the corpuscles had been added.

TABLE XVIII.—*Human serum freed from antihorse amboceptor titrated against guinea pig corpuscles.*

	Serum.			
	0.4	0.2	0.1	0.05
Whole human serum	+	+	+	±
Human serum freed from antihorse amboceptor	+	+	+	±

Table XVIII shows that removing the antihorse amboceptor did not appreciably disturb the antiguinea pig amboceptor.

Antihorse amboceptor in serum freed from antiguinea pig amboceptor.—After having removed the natural antiguinea pig

amboceptor, the serum was tested for hæmolytic power against the corpuscles of the horse. Two sets of tubes, designated as A and B, were used. Each set contained four tubes marked 1, 2, 3, and 4, respectively. In set A the whole human serum was titrated. Tubes 1, 2, 3, and 4 received 0.4, 0.2, 0.1, and 0.05 cubic centimeter of serum, respectively. To each tube was added 0.5 cubic centimeter of 4 per cent suspension of horse corpuscles and enough physiologic salt solution to bring the total quantity up to 2.5 cubic centimeters. In set B the serum freed from antiguinea pig amboceptor was tested for hæmolytic power against horse corpuscles. The serum was used in quantities of 0.4, 0.2, 0.1, and 0.05 cubic centimeter, and horse corpuscles and salt solution were added as in set A. All tubes were shaken, placed in the incubator at 37°C. for one hour, and removed to room temperature; the results were read about three hours after the corpuscles had been added.

TABLE XIX.—*Antihorse amboceptor in serum freed from antiguinea pig amboceptor.*

Set—	Serum.			
	0.4	0.2	0.1	0.05
A	+	+	tr	0
B	+	+	0	0

Table XIX shows the results obtained. Removing the natural antiguinea pig amboceptor left the natural antihorse amboceptor practically undisturbed.

CONCLUSION

Unheated, fresh human serum dissolves the red blood corpuscles of the sheep better than it dissolves those of the guinea pig, goat, horse, or rabbit.

For guinea pig corpuscles the hæmolytic power of fresh, unheated human serum is slightly higher than for goat corpuscles.

The hæmolytic power of fresh, unheated human serum is lower for the corpuscles of the rabbit than for the corpuscles of sheep, goat, horse, or guinea pig.

A fresh, unheated human serum may dissolve the corpuscles of one horse and be inactive against the corpuscles of another horse. The corpuscles differ in resistance toward hæmolysins.

The natural antisheep and antigoat amboceptors can readily be reactivated with guinea pig complement.

The natural antirabbit amboceptor remained nearly inactive when 0.05 cubic centimeter of guinea pig complement serum was used; larger quantities of guinea pig serum reactivated the amboceptor well.

Guinea pig serum is slightly hæmolytic for rabbit corpuscles.

Guinea pig complement, even when used in large doses, does not readily reactivate the natural antihorse amboceptor. This amboceptor can be readily reactivated with human complement.

Guinea pig complement does not reactivate the natural antiguinea pig amboceptor.

Natural antiguinea pig amboceptor can readily be reactivated with human complement.

Natural antiguinea pig amboceptor is easily removed from human serum.

Natural antihorse amboceptor is not easily removed from human serum.

Fresh, unheated human serum left in contact with guinea pig corpuscles for more than three hours loses the power to reactivate the antiguinea pig amboceptor.

Natural antihorse amboceptor can be removed from human serum without disturbing the natural antiguinea pig amboceptor.

Natural antiguinea pig amboceptor can be removed from human serum without disturbing the natural antihorse amboceptor.

AN EXPERIMENTAL STUDY ON THE USE OF APOMORPHINE TO REMOVE FOREIGN BODIES FROM THE RESPIRATORY PASSAGES [1]

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TWO PLATES AND 1 TEXT FIGURE

The use of apomorphine to remove foreign bodies from the respiratory passages is still mentioned in standard textbooks of pharmacology.[2] It is claimed that coincidentally with the act of vomiting caused by apomorphine violent movements of expiration are produced [3] which expel or at least facilitate the expulsion of the foreign body from the respiratory passages. We have not been able to find any reference in the literature that this manner of action of apomorphine has been experimentally established. The present investigation was carried out in order to test whether or not apomorphine exerts such an action.

THE IRRITABILITY OF THE VOMITING CENTER IN ASPHYXIA

As we are interested in the emetic action of apomorphine when there is obstruction to the respiration, it becomes important first to determine the irritability of the vomiting center in varying degrees of asphyxia. Because of the ready response of the vomiting center to intramuscular injections of apomorphine, dogs were used in all the experiments. Under light ether anæsthesia the tracheal cannula was inserted into the trachea through a short incision in the anterior median line of the neck. The animal was allowed to recover from the influence of anæsthesia. About two hours later different degrees of obstruction to the passage of air into the trachea were produced by

[1] Received for publication January 28, 1916.

[2] Cushny, Text-book of Pharmacology and Therapeutics. 5th ed., Lea & Febiger, Philadelphia & New York (1910), 242. Sollmann, Text-book of Phamarcology. 2d ed., W. B. Saunders Co., Philadelphia & London (1908), 313. Wood, Pharmacology and Therapeutics. J. B. Lippincott Co., Philadelphia & London (1912), 244.

[3] Cushny, loc. cit.

placing a tight clamp on the rubber tubing connected with the free end of the tracheal cannula and by connecting the rubber tube with short pieces of glass tubing whose diameters at one end had been flamed to about 0.9 and 1.5 millimeters, respectively. Apomorphine hydrochloride (0.1 cubic centimeter of a 2 per cent solution per kilogram of body weight) was injected intramuscularly at varying intervals from the commencement of respiratory obstruction. When the occlusion was complete, or when the animals were made to breathe through a circular opening of about 0.9 millimeter in diameter, the vomiting center became quickly paralyzed and apomorphine failed to cause vomiting, even if it was injected at the same time that the respiratory obstruction was accomplished. However, when the animals were made to breathe through a larger opening of approximately 1.5 millimeters in diameter, the vomiting center remained irritable to apomorphine. This observation was not continued longer than two hours, but judging from the behavior of the animals at the end of the observation, the vomiting center would probably have remained irritable even after hours of this degree of asphyxiation. These results are illustrated in the subjoined protocols:

Protocol 1. November 5, 1915. Male dog, 12.05 kilograms.

10.25 a. m.	The dog was put under light ether anæsthesia, the tracheal cannula inserted, the wound closed, and the anæsthesia discontinued.
2.55:30 p. m.	The dog had recovered from the anæsthesia. A clamp was placed tightly on the rubber tube connected with the tracheal cannula.
2.55:50 p. m.	Apomorphine hydrochloride (0.1 cubic centimeter of a 2 per cent solution per kilogram) was injected intramuscularly in the gluteal region.
2.56 p. m.	Retching occurred, and the dog fell to the floor.
3.00 p. m.	The heartbeat was not palpable.

Protocol 5. December 21, 1915. Female dog, 5.27 kilograms.

11.45 a. m.	The tracheal cannula was inserted, the wound sewed, and etherization discontinued.
2.02 p. m.	The tracheal cannula was connected to a glass tube with an opening of about 0.9 millimeter in diameter. Apomorphine hydrochloride (0.1 cubic centimeter of a 2 per cent solution per kilogram) was injected intramuscularly.
2.04:30 p. m.	Retching occurred, and the dog fell to the floor.
2.08 p.m.	The dog was unconscious, the muscles were relaxed, and the limbs dropped limp when lifted. The femoral pulse was hardly perceptible.

Protocol 7. December 22, 1915. Male dog, 9.05 kilograms.

11.15 a. m.	The tracheal cannula was inserted. The dog was allowed to recover from the ether anæsthesia.
1.34:30 p. m.	The tracheal cannula was connected to a glass tube with an opening of about 1.5 millimeters in diameter.
3.35 p.m.	The dog lay quietly on the floor. There was slight dyspnœa, but unnoticeable cyanosis (in the tongue). Apomorphine hydrochloride (0.1 cubic centimeter of a 2 per cent solution per kilogram) was injected intramuscularly.
3.36 p. m.	Nausea was observed.
3.36:30 p. m.	The dog vomited. At 3.37, 3.37:30, and 3.38 he vomited again, once each time.
3.39 p. m.	The dog was chloroformed.

When nonanæsthetized dogs are asphyxiated by shutting off the air from the trachea, retching commonly occurs within the first three minutes without the administration of apomorphine; except in rare cases, the animals die without vomiting. It appears that the vomiting center shares, with the other medullary centers (respiratory, vasomotor, and vagus), the transient stimulation followed by paralysis brought about by asphyxia. In three intramuscular injections of apomorphine hydrochloride (0.1 cubic centimeter of a 2 per cent solution per kilogram of body weight) into three normal dogs, the interval between the injection and the onset of vomiting averaged two minutes and twenty seconds, and in no case was it shorter than two minutes and twelve seconds. The absence of vomiting in experiments 1 and 5 does not necessarily indicate that the vomiting mechanism (center, nerve fibers, nerve endings, or muscles) was paralyzed within two and a half minutes of respiratory obstruction. We have observed in another animal that the femoral pulse was reduced from 78 to 48 beats per minute and became weaker and irregular at the end of the first minute of almost complete tracheal occlusion. It is, therefore, possible that sluggish circulation occurred very early in experiments 1 and 5 and that emesis would have taken place had apomorphine reached the vomiting center in the usual time. Although the vomiting mechanism is readily thrown out of function by complete asphyxia, it is interesting to note that in partial asphyxia, such as is produced by reducing the tracheal opening to 1.5 millimeters in diameter, it not only survives two hours, but it appears more irritable and consequently responds more quickly to apomorphine, as was observed in experiment 7 and in two other experiments.

WILL THE ADMINISTRATION OF APHOMORPHINE FACILITATE THE EXPULSION OF FOREIGN BODIES FROM THE TRACHEA?

Since the vomiting center remains irritable even in the presence of marked respiratory obstruction, we proceeded to test if the administration of apomorphine would facilitate the expulsion of a foreign body from the trachea. With the hope of accomplishing this end, cylinders of reddened agar jelly cut by means of different-sized cork borers were blown with the aid of glass tubing into the trachea of dogs under light ether anæsthesia. The animals were then allowed to come out from under the influence of the anæsthetic and given injections of apomorphine. Seven experiments of this type were performed. One dog died of asphyxia before the administration of apomorphine, and the agar plug, on post-mortem examination, was found at the openings of the two bronchi. Protocol 18 will serve as an illustration of the other six experiments.

Protocol 18. August 25, 1915. Male dog, 8.05 kilograms.

10.35 a. m. Etherization was commenced.
11.02 a. m. An agar cylinder, 6 millimeters in diameter by 40 millimeters long, was blown into the trachea with the aid of a glass tube inserted past the vocal cords.
11.03 a. m. The dog was released; he was restless and had moderate dyspnœa.
11.04 a. m. A segment of agar cylinder, 7 millimeters long, was expelled by forced expiration.
11.06 a. m. The animal was less restless. Respiration was noisy.
11.08 a. m. Four cubic centimeters of a 2 per cent solution of apomorphine hydrochloride were injected hypodermically.
11.11 a. m. Emesis occurred. The vomitus contained agar cylinder about 10 millimeters long.
11.20 a. m. Respiration was still noisy. Chloroform was injected directly into the heart. Autopsy showed no traumatism of the larynx and no agar in the trachea nor bronchi. The remaining segment of the agar was found in the stomach.

From these six experiments it would appear that apomorphine causes expulsion of agar cylinders introduced into the trachea. However, we have not been able to determine that they were still in the trachea at the time of vomiting. In 15 control experiments, where the animals were chloroformed before receiving apomorphine, but still showed difficulty of respiration, the agar was found in the trachea of only two dogs, while in the others it was found in the stomach. The results of these experiments, therefore, are unsatisfactory and not conclusive.

RESPIRATORY PRESSURE AND CONDITION OF THE GLOTTIS DURING VOMITING

According to the manner of action ascribed to apomorphine as an agent to remove foreign bodies from the respiratory passages, it must cause a rise of the intrapulmonic pressure and opening of the glottis, or, in other words, a forced expiration during the act of vomiting. The determination, therefore, of the intrapulmonic pressure and the condition of the glottis during emesis should furnish important information relative to the mode of action of apomorphine under consideration.

The intrapulmonic pressure was taken from a tracheal cannula and recorded as follows: One limb of a Y-tube was connected with the tracheal cannula by means of rubber tubing; the second limb was connected with a mercury manometer whose float was adjusted to write on a slow drum; and to the third limb was attached a short piece of rubber tubing so arranged that it could be readily closed or opened when desired. Plate I, fig. 1, is a tracing taken during emesis produced by apomorphine when the third limb of the Y-tube was closed before the beginning of one of the deep inspirations at the commencement of vomiting. The pressure oscillated about the zero line, but in the expulsion time—the time which elapses from the beginning of the convulsive contraction of the abdominal wall to the appearance of vomitus in the mouth—it reached a high level. When the pressure was taken from the perpendicular limb of a T-tube whose horizontal limb was inserted through the median cervical line into the trachea so that it did not interfere with the mechanism of the glottis, the curves obtained were almost identical with Plate I, fig. 1. The curves obtained in this way are illustrated in Plate II, fig. 2. The pressure in this case sank much lower below the zero line during the deep inspirations, and as the rise of pressure was relatively lower, the latter might have been simple rebounds of the mercury in the manometer and not actual increased pressure. This could not, however, be the chief cause; otherwise the waves should progressively diminish. This they did not do. From the standpoint of the mechanism of vomiting, attention is called to the late appearance of the vomitus in the mouth in relation to the beginning of the rise of intrapulmonic pressure. The time relation of these two events seems to indicate that the œsophagus is probably not subjected to a negative pressure

during the passage of vomitus through it as some authors are inclined to believe.[4]

The similarity of the curves, Plate I, fig. 1, and Plate II, fig. 2, affords strong evidence that the glottis closes in the act of vomiting.[5] We have observed, also, when the trachea is connected with a mercury manometer and the dog is allowed to respire through a side tube the terminal opening of which has the combined area of the two nostrils of the animal, that vomiting does not cause a rise of intrapulmonic pressure. The increased pressure noted when the trachea is not occluded, then, must be associated with partial or complete closure of the glottis. On the ingenious suggestion of Prof. R. B. Gibson, of the College of Medicine and Surgery of this University, the condition of the glottis during emesis was further studies as follows: A tracheal cannula was inserted into the trachea—opening directed toward the glottis—and by a Y-tube was connected with a mercury manometer and water-air-pressure pump as shown in fig. 1. If the water is turned on, a current of air flows from the pump through the Y-tube, tracheal cannula, and out of the glottis during quiet respiration, without raising the mercury in the manometer, but if the lumen of the rubber tube connecting the tracheal cannula with the Y-tube be occluded, the current of air is deflected toward the manometer and the mercury rises, falling partially after about four seconds, and then oscillates about this new level till the occlusion is removed. An illustration of this is Plate II, fig. 1. Plate I, fig. 2, shows the curves obtained when the dog vomited. The rise of pressure indicates an obstruction to the passage of air through the naso-laryngo-tracheal passages, and as under the condition of the experiment the dog can accomplish this only by closing the glottis, it serves, therefore, as evidence that this organ closes in the act of vomiting.

The closure of the glottis may persist for ten seconds. Its tracing, when taken simultaneously with that of the intrapulmonic pressure, shows that the glottis stays closed until about the end of vomiting. Whether the glottis opens before or after the increased intrapulmonic pressure has returned to normal, we have not satisfactorily ascertained. The tracings which we could only take on slowly moving drums are not conclusive. However, our objective observation on the movement of air in the trachea

[4] See Howell, Text-book of Physiology. 6th ed., W. B. Saunders Co., Philadelphia & London (1915), 736.

[5] "Act of vomiting" is used here and in the following pages to designate the series of events beginning with the first deep inspiration to the appearance of vomitus in the mouth.

during emesis furnishes strong evidence that the glottis remains closed after the increased intrapulmonic pressure has returned to its level before the onset of vomiting. In order to carry on this observation, two cannulæ were inserted into the trachea, one toward the glottis and the other toward the lungs. The free ends of the cannulæ were joined to a piece of glass tubing, so

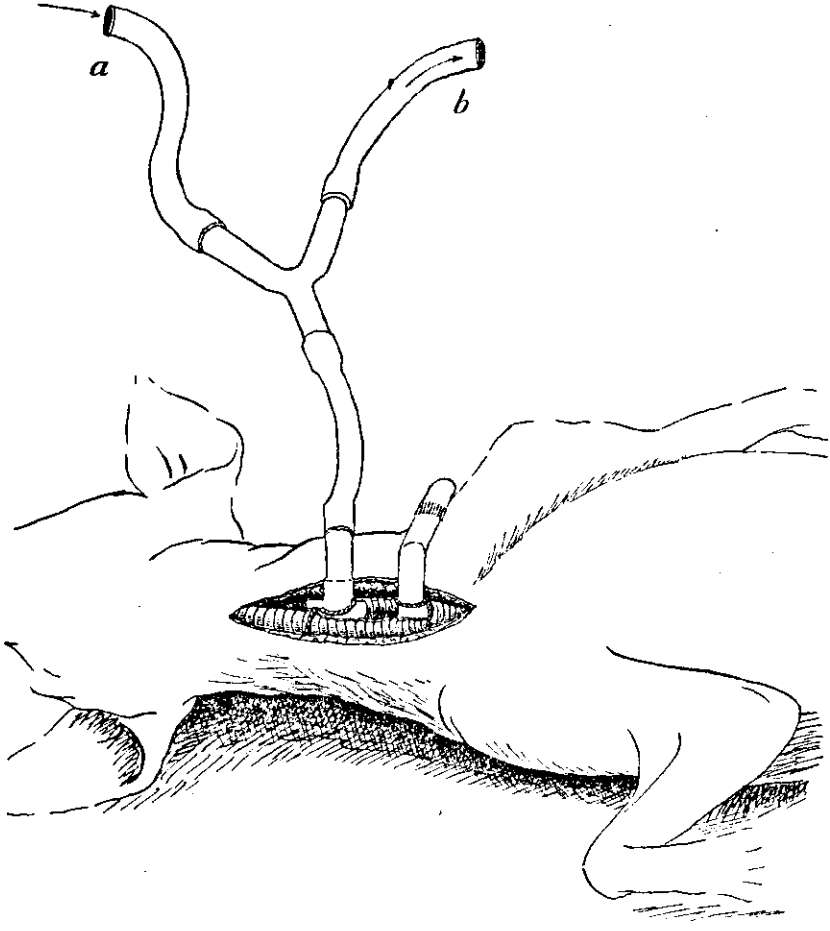


FIG. 1. Shows the connection of the glottis to *b*, the mercury manometer, and to *a*, water-air-pressure pump.

that when the preparation was completed the respired air passed from the lungs through one cannula, through the glass tubing, through the other cannula, and out through the nostrils. During quiet respiration the cork cylinder previously inserted into the glass tubing moved toward the glottis with each expiration and toward the lungs with each inspiration; however, as soon as emesis set in, its movement ceased till the vomitus appeared in

the mouth, at which instant the intrapulmonic pressure must have already fallen to about normal, as Plate I, fig. 1, and Plate II, fig. 2, seem to indicate. When the cork began to move, it did so slowly, and its movement did not exhibit any indication that extra forcible expiration occurred following the act of vomiting.[6] The absence of air current in the respiratory passages during the act of vomiting is shown most decisively if emesis sets in when the cork is about in the middle portion of the glass tube.

The foregoing results point conclusively to the impossibility of removing foreign bodies from the trachea by the use of apomorphine. It seems, moreover, that a foreign body in the respiratory passages below the larynx may, in reality, be driven farther in during the early stage of vomiting because of the descent of the diaphragm and closure of the glottis, which in turn give rise to the rarefaction of the air in the thoracic cavity and a rushing of the air into the deeper portion of the lungs. When tenacious mucous plugs are present in the bronchioles, this may be more than counterbalanced by the stimulating effect of apomorphine on the secretion [7] and peristalsis [8] of the bronchioles, which may loosen and facilitate the expectoration of the plugs after vomiting.

SUMMARY AND CONCLUSIONS

Transient stimulation followed by paralysis of the vomiting center occurs when nonanæsthetized dogs are asphyxiated by shutting off the air from the trachea.

Partial asphyxia, such as is produced by reducing the lumen of the trachea to a circular opening of about 1.5 millimeters in diameter, shortens the time required for the emetic action of apomorphine. This is due, presumably, to the increased irritability of the vomiting center to apomorphine.

The intrapulmonic pressure is raised by the convulsive contraction of the abdominal wall which occurs during vomiting, and the rise of pressure seems to begin before the passage of vomitus through the œsophagus.

The glottis remains closed during the act of vomiting, as shown by the method above described. This conclusion is fur-

[6] Cf. Starling, Schäfer's Text-book of Physiology. Young J. Pentland, Edingburg & London, (1898) 2, 325.

[7] Henderson and Taylor, *Journ. Pharmacol. & Expt. Therap.* (1911–12), 2, 153.

[8] Meyer and Gottlieb, Die Experimentalle Pharmakologie. Dritte Auflage. Urban & Schwarzenberg, Berlin und Wien (1914), 329.

ther confirmed by the observation that no expiration occurs during the act of vomiting.

A strong expiratory effort is not produced immediately after the expulsion of the vomitus.

The administration of apomorphine cannot facilitate the removal of foreign bodies from the trachea.

ACKNOWLEDGMENT

We wish to acknowledge our indebtedness to Prof. R. B. Gibson for reading the manuscript of this paper and for many kind and valuable suggestions made by him during the progress of the experiments.

ILLUSTRATIONS

PLATE I

FIG. 1. Intrapulmonic pressure during apomorphine emesis with the tracheal cannula closed. *C* indicates closure of the third limb of the Y-tube; 1-5, deep inspirations at the beginning of vomiting; 6-7, expulsion time; *O*, opening of the third limb of the Y-tube; *Z*, zero line.

2. Pressure produced by the water-air pump when the dog vomits. 1, beginning of vomiting; 2, end of vomiting; *W-W*, waves due to swallowing of vomitus; *w*, waves due to oscillations of mercury in seeking its normal level; *z*, zero line.

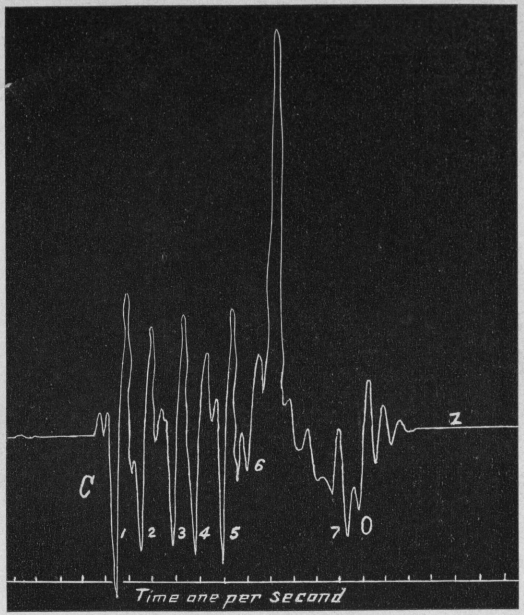
PLATE II

FIG. 1. Pressure produced by the water-air pump when the escape of air through the glottis is artificially prevented. 1, occlusion was produced between the tracheal cannula and Y-tube; 2, occlusion removed; *w*, waves due to oscillations of mercury in seeking its normal level; *z*, zero line.

2. Intrapulmonic pressure during apomorphine emesis with the trachea not occluded. 1-8 indicate deep inspirations at the beginning of vomiting; 9, beginning of contraction of abdominal wall; *v*, appearance of vomitus in the mouth.

TEXT FIGURE

FIG. 1. Shows the connection of the glottis to *b*, the mercury manometer, and to *a*, the water-air-pressure pump.



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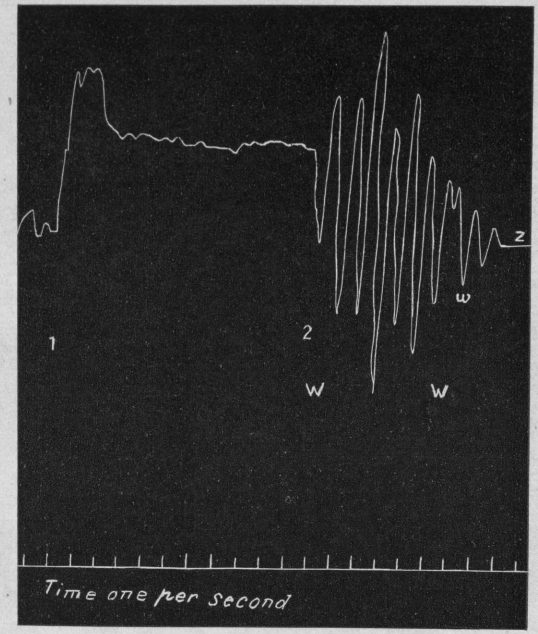
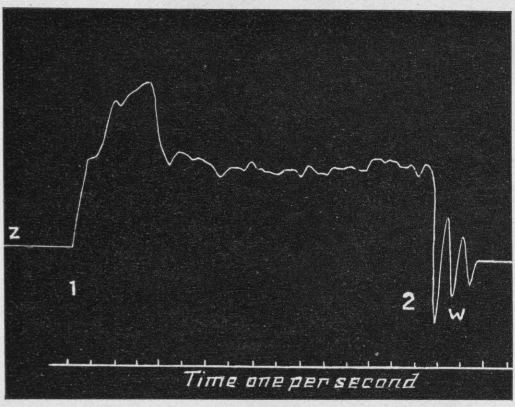


Fig. 2. Pressure produced by the water-air pump when the dog vomits. 1, beginning of vomiting; 2, end of vomiting; W–W, waves due to swallowing of vomitus; w, waves due to oscillations of mercury in seeking its normal level; z, zero line.

PLATE I.



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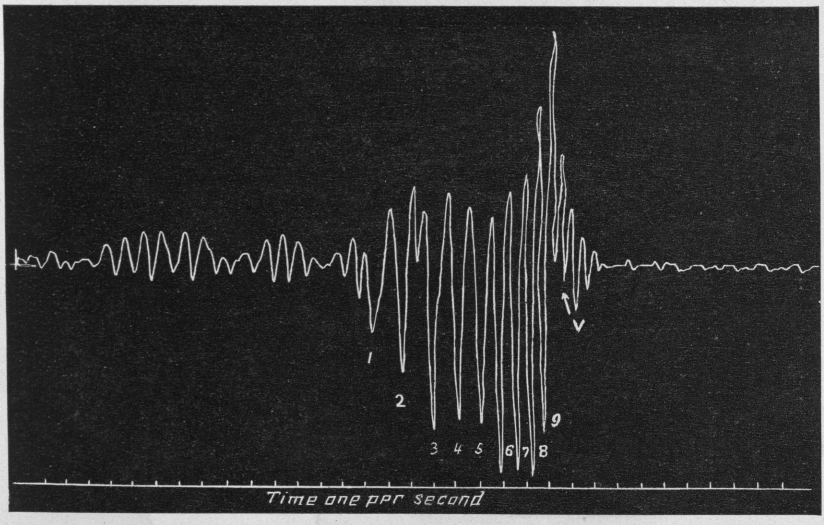

Fig. 2. Intrapulmonic pressure during apomorphine emesis with the trachea not occluded. 1–8 indicate deep inspirations at the beginning of vomiting; 9, beginning of contraction of abdominal wall; v, appearance of vomitus in the mouth.

PLATE II.